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Original Article

Drug Abuse of Professional Drivers: Experience from Referral Dope Test

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Abstract

In Bangladesh, drug abuse is one of the dangerous problems among drivers that hamper road safety and make government worry. In that context, this cross-sectional study was conducted in National Institute of Laboratory Medicine and Referral Center (NILMRC) and aims to determine the proportion of drug abuser among professional drivers in Bangladesh. This study also addressed to recognize the various types of abused drugs, the pattern and the trend of drug abusers during study period. Data were collected from online data server of NILMRC during the period of July to December 2022. Bangladesh Road Transport Authority (BRTA) customarily refers urine samples of drivers to NILMRC for dope test. Dope tests were performed in immunochromatographic test (ICT) device and semi-auto analyzer. Dope results and demographic data of drivers were well-kept-up in online server for electronic transfer and use of data. Commonly abused drugs in Bangladesh such as, cannabinoids, amphetamines, opiates, benzodiazepines and alcohol were assessed. A total of 70866 drivers had been tested for six months of study period among them 2720 (3.81%) were found dope test positive. Considering monthly trends from July to December 2022 the highest incidence were found in December where 473 (4.58%) of 10323 drivers were dope test positive; however in November 639 (4.56%) of 14,017, in September 682 (4.07%) of 16757, in October 551(3.62%) of 15221, in August 320 (3.42%) of 9348, then less incidence were found in July 55 (1.05%) of 5200 drivers had been tested positive. There was an increase number of test positive cases were detected during the period from July to December. The highest number drug abuser that is more than two-third (70%) of drivers were found among young adult age group (25-44) years. The five types of abused drugs were tested; among those the highest proportion (91.5%) of abused drug was Cannabinoids, then Benzodiazepines was 5.5%, Amphetamine was 1.3%, Opiates was 1% and Alcohol was 0.8%. No female drivers were found test positive. Cannabinoids was the most common drug of abuse among drivers referred by BRTA, Bangladesh. During this study, there was challenge to different kind illegal means. If the illegal means could be resolute then the data of positive finding would be higher than this finding. So implementation online registration, proper identification by finger print or retinal scanning and providing online reports may minimize the illegal means.

Keywords: *Drug abuse; BRTA; drivers; cannabinoids; benzodiazepines NILMRC.*

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INTRODUCTION

Road traffic accident became common phenomenon in Bangladesh. It is well established that severe road traffic accidents are related with drug abused drivers.[1,2] The fact is estimated that near about 6 million people in Bangladesh are drug addicted and about 80 percent of the drug addicts are adolescents and young men of 15 to 30 years of age.[3] Drug or substance abuse refers to the using of any psychoactive substance, illicit or medically prescribed drugs.[4] These drugs such as: amphetamines, cannabis, opioids, benzodiazepines and alcohol. Drug abuse alters the brain functions which are necessary for harmless driving.[5]

Cannabis, the second most commonly used drug in the world after alcohol[6] is the major cause of impaired driving.

Although no specific study is available about the number of drug-addicted drivers in Bangladesh, according to transport owners, a large number of Dhaka's drivers and their assistants, who operate around 50,000 public transport vehicles, are drug addicts. Private organizations have found the rate of drugged drivers stands around 80%. Drivers claim, they need drugs to maintain concentration through high temperatures, constant shouting by passengers, long working hours. Drugs help them tackle these pressures.[7] Proper strategies and planning should be developed to identify drug abused drivers and correct them for safe driving.

In this background, the prime minister of Bangladesh directed Bangladesh Road Transport Authority (BRTA) to have the DOPE test certificate mandatory for all the professional drivers prior to issuing or renewing their driving license. To implement that order, BRTA started advising dope test since 30 January'22 for drivers. No previous nation-wide study for BRTA drivers with large and representative samples of the population has been conducted yet in Bangladesh. So, with all of these relevant findings, this study aimed to determine the prevalence of drug abuse and notify the types of drug abuse in drivers of Bangladesh.

MATERIALS AND METHODS

This cross sectional study was conducted at Department of Biochemistry, NILMRC, Sher-e-Bangla Nagar, Dhaka from July to December 2022**.** During that period 70866 drivers were referred from BRTA to NILMRC for dope test for the purpose of issuance of driving license. and at attended in OPD We did not include people who came for Dope test for job purpose not referred from BRTA. All the referred drivers attended in out patients department (OPD) and after recorded their personal information at OPD they were then sent to laboratory for their dope test.

Laboratory assay

All laboratory investigations were done in the department of Biochemistry, NILMRC, Dhaka and the procedure of dope test was done from urine sample using ICT method by semi-auto analyzer.

Laboratory Procedures

Specimen collection (Collection of urine): After checking national identification card (NID) and reference paper from BRTA, only drivers were allowed to enter into guarded area for registration; other peoples such as dope test for job purpose or not referred from BRTA were excluded from this study. After registration one sterile urine tube was supplied for each driver and instruction was given for urine collection into urine tube. Urine samples of drivers were collected in a toilet without a sink, water flush, detergents, or any other potential adulterants.

Test Procedure: Urine specimens were screened for amphetamine, benzodiazepines, cannabinoids, opiates and alcohol using multi-drugs (5 drugs) rapid test cassette (urine). The multi-drugs 5 drugs rapid test cassette (urine) is a rapid chromatographic immunoassay for the qualitative detection of drugs and drug metabolites in urine. They are rapid urine screening test based on the principle of competitive binding. Positive samples were run into the Indico Plus Semi Auto analyzer machine for the quantitative assay. This is based on photometric method. Positive samples were kept preserved at twenty degree Celsius (20^0C) refrigerator for one month for any kind of necessity. Universal precaution was obtained. gloves, laboratory coat were worn when handling urine sample. Contaminated urine tube, gloves were placed in a biohazard bag. Disinfection of all working surfaces was done. Washing hands thoroughly was done after removal of personal protective devices used in handling specimens and kit reagents.

Statistical Analysis

Data was collected from hospital online server. Data editing, clearing and analysis were done by statistical package for social science (SPSS). Data and result were presented in the form of tables and graph where applicable.

Ethical Consideration

This study is approved by Institutional Review Board (IRB) of NILMRC, Dhaka for ethical clearance.

RESULTS

A total of 70866 drivers had been tested for dope at NILMRC from July to December 2022, and among them 2691 (3.80%) were found positive. Among these test, number of female drivers were 203 (0.28%), but there were no test positive female drivers.

Table I illustrates the monthly distribution of dope tests and positive cases from July to December 2022. In July, 5200 drivers had been tested among them 55 (1.05%) were positive; in August, September, October, November and December 9348, 16757, 15,221, 14,017 and 10,323 drivers had been tested; among them 320 (3.42%), 682 (4.06%), 551 (3.61%), 639 (4.55%) and 473 (4.58%) were positive respectively.

Table- I: Monthly distribution of dope tests and positive cases from July to December 2022

Month	Total Tests	Positive (%)	P value
July	5200	55 (1.05%)	
August	9348	320 (3.42%)	
September	16757	682 (4.07%)	<0.001*
October	15221	551(3.62%)	
November	14017	639 (4.56%)	
December	10323	473 (4.58%)	
Total	70866	2720	

Table II shows the age distribution of dope test positive drivers. Among them, 285 (10.60%), 952 (35.37%), 926 (34.41%), 390 (14.50%), 111 (4.12%) and 27 (1.00%) test positive drivers were found in age group <25, 25-34, 35-44, 45-54, 55-64 and >65 years respectively. The mean age of dope test positive drivers is 33.31±8.62 years.

Table- I1: Age group distribution with tested positive number (n= 2691)

Age Group (years)	Number	Percent (%)
<25	285	10.60
25-34	952	35.37
35-44	926	34.41
45-54	390	14.50
55-64	111	4.12
>65	27	1.00
Total	**2691**	**100**

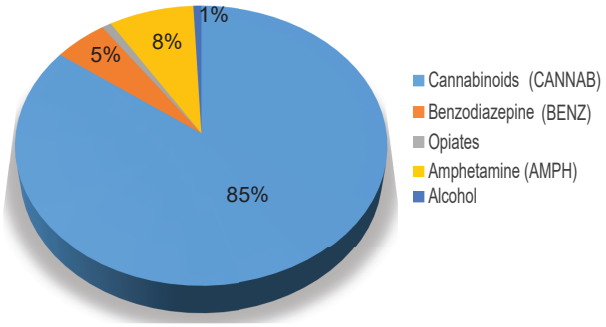

Figure-1: *Distribution of various types of drugs abused by drivers (n= 2691)*

Figure 1 states the distribution of various types of drugs abused by drivers; out of the 70866, 2691 drivers were dope test positive, among the dope positive drivers 2461 (91.5%) were positive to Cannabinoids (CANNAB); followed by 148 (5.5%) Benzodiazepine (BENZ), 34 (1.2%) Amphetamine (AMPH), 26 (1.0%) Opiates and 22 (0.8%) were positive to Alcohol.

Table III comprises the distribution of types of abused drug among age group of test positive drivers; the types of abused drug Cannabinoids (CANNAB), Benzodiazepine (BENZ), Opiates, Amphetamine (AMPH) and Alcohol were found among 2461 (91.5%), 148 (5.5%), 26 (1.00%), 34 (1.2%) and 22 (0.8%) drivers. Cannabinoids abused were found in 261 (9.70%), 871 (32.37%), 847 (31.48%), 357 (13.27%), 101 (3.75%) and 24 (0.89%) drivers among the age group of <25, 25-34, 35-44, 45-54, 55-64 and >65 years respectively.

Table- III: Distribution of types of abused drug among age group (n= 2691)

Age Group	Cannabinoids (CANNAB)	Benzodiazepine (BENZ)	Opiates	Amphetamine (AMPH)	Alcohol
<25	261 (9.70%)	15 (0.56%)	2 (0.07%)	4 (0.15%)	3 (0.11%)
25-34	871 (32.37%)	53 (1.97%)	10 (0.37%)	13 (0.48%)	5 (0.19%)
35-44	847 (31.48%)	51 (1.90%)	9 (0.33%)	12 (0.45%)	7 (0.26%)
45-54	357 (13.27%)	21 (0.78%)	4 (0.15%)	4 (0.15%)	4 (0.15%)
55-64	101 (3.75%)	6 (0.22%)	1 (0.04%)	1 (0.04%)	2 (0.07%)
>65	24 (0.89%)	2 (0.07%)	0 (0.00%)	0 (0.00%)	1 (0.04%)
Total	2461 (91.5%)	148 (5.5%)	26 (1.00%)	34 (1.2%)	22 (0.8%)

DISCUSSION

In the present study, our results revealed that 3.80% of the drivers in Bangladesh are drug abusers. Highest percentage of drug abuse are in the age group (25-34) years which is 35.37% followed by the age group of (35-44) which is 34.41%. This is an alarming sign because this age group is the most energetic and creative group of people who can contribute to the economy and development of Bangladesh.

This is in agreement with a study which revealed that the age range of 25-34 years of the commercial bus drivers are more subjected to drug abuse.[8] There is another study among 427 drivers admitted to Alexandria Main University Hospital after road traffic accidents. They revealed that the highest age group was 35-45 years old.[9] Moreover, a study was done in America in 2013 found that the high rate of drug abuse (about 30%) are in young adults.[10] While these results are incomparable to a study who have been showed that highest age group was 18-30 years.[11] This difference may be due to wide variation and large number of drivers used in our study.

The results of this study showed that cannabinoids were the most common drug of abuse among drivers referred from BRTA. This may be attributed to widespread of cannabis which it is easily gained and cheaper than the other drugs. Our findings correlate a study who reported that cannabis is the most common abused drug among the cab-drivers.[12] Another study revealed that the combination of cannabis and tramadol has higher prevalence than other combination.[13] The present results are at variance to a study who reported that the commercial bus drivers subjected to drug abuse with low-price and easily obtained to them such as tramadol.[8]

Another study recorded that opioids are the most frequently abused drug among the Iranian drivers followed by cannabinoids.[13] A study has concluded that alcohol abuse stands for the second most common drug abuse though its incidence is low.[14] It is consistent with the WHO report, where stated that, prevalence of the road traffic accidents increases with alcohol (above 0.04 g/dl BAC).[15] In our study, we have found alcohol as a least percentage which may not be the real picture of driver drug abuse habit. The reason behind this, urine test cannot detect alcohol 48 hours after consumption of alcohol.

In our study, no female drivers were found to be positive. This is consistent with other studies where no female drivers are recorded to be positive.

CONCLUSSIONS

In this first large-scale study on drug abuse among drivers in Bangladesh, a significant proportion of drivers were found to be abuser in Bangladesh. Cannabinoids was the most common drug of abuse among drivers referred by BRTA in Bangladesh. Number of positive drug abuser was increased during the period of study.

Limitations:

The maximum number of tests procedure were ICT method due to the fixation of rate from government. Only the positive tests from ICT were reanalyzed in semi- auto analyzer; if all the tests could have done in auto-analyzer, then the report would have been more authentic but at the same time expensive. This study was conducted in only one center and among professional drivers which merely represents whole country.

Recommendations:

This study observed that the proportion of alcohol abuser is very low, the fact behind it is, urine test could detect alcohol between 12 - 48 hours after drinking. So, road side breath test and tests for alcohol in hair, sweat, saliva or blood should be performed for alcohol abuser. It has also been observed that the number of positive drug abuser were increased in different months during the period of study and it may be due to manipulation of results for corruption. For authentic report production, this manipulation may be minimized by immediate implementation of online registration, biometric technique for identification and patient portal system. Nationwide large scale study among all categories drivers would reflect the real scenario and to take proper steps to identify them and reduce the incidence of road traffic accident.

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Original Article

Genetic Characterization of *Aspergillus flavus* and *Saccharomyces cerevisae* in Tracheobronchial Phlegm of HIV-infected Patients on Antiretroviral Therapy in Jalingo, Nigeria

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Abstract

Fungal infections are among the diverse respiratory tract pathogens and account for a proportion of community acquired and nosocomial pneumonias thereby generating concerns particularly in immunocompromised patients. This study aims to genomically extract and sequence fungal DNA using the Basic Local Alignment Search Tool (BLAST) technology for their definitive and confirmatory identification thereby easing off the obstacles in their diagnosis due to similar appearance in their colony and morphology and ultimately determining their infection rates in persons receiving antiretoviral therapy against HIV. Sputa of 100 HIV infected out-patients of a Medical Centre in Jalingo, on anti-retroviral therapy were cultured on Sabouraud Dextrose Agar to isolate fungi species and assess their prevalence and distribution. Fungal colonies characterized culturally and biochemically as Aspergillus flavus and Aspergillus niger were subjected to the BLAST, and the similarities with the biological sequences in the National Center for Biotechnology Information (NCBI) database queried. There were observational variances in the colonial and microscopic appearances of A. flavus and A. niger on Sabouraud Dextrose Agar (SDA) plates and the microscope respectively. The similarities between the queried and biological sequences in the NCBI database, was almost all (99.7%) thus confirming their identity as Aspergillus flavus and Saccharomyces cerevisiae. The overall prevalence of fungi infection was 79.0%. More males (80.0%) than females (78.3%) were infected. In both sexes, fungi were most (92.9%) frequently isolated in patients that were between 30 and 39 years and least (64.7%) in those between 15 and 29 years. There was no established pattern (sex- and age- relatedness) of non-concomitant A. flavus and S. cerevisiae in fifty-seven male and female patients in six age categories even though A. flavus occurred more (48.1%) than S. cerevisae (24.1%) and the prevalence was higher (61.4%) in females than in males (38.6%). The high prevalence of these fungi in the study population, with or without symptoms of cough or fungal disease, mandates an early screening of such infected persons so as to reduce further complications and improve treatment.

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Keywords: *Genetic, characterization, Aspergillus flavus, Saccharomyces cerevisae, HIV, tracheobronchial phlegm, anti-retrovirals*

INTRODUCTION

The human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome (AIDS)[1]. Fungi may colonize human body sites with no manifestation of disease.[2,3] Respiratory infection occurs when spores or conidia are inhaled or a latent infection is reactivated.[2] Endemic or opportunistic infections in persons with HIV increase disease morbidity.[4,5,6]

Aspergillus, the aetiological agent of aspergillosis, is commonly transmitted to humans through inhalation of spores.[2] The incubation period is unclear since the effects of spore inhalation mostly depend on an individual's immunological state.[2] Pulmonary aspergillosis is a global healthcare concern because, if left untreated and undiagnosed in immunocompromised individuals, could rapidly progress to other organs and result in lethal invasive illnesses.[2,7] *A. fumigatus* stands out for its frequency in humans (about 90% of cases) and for being substantially responsible for the rise in invasive pulmonary aspergillosis (IPA) among immunocompromised individuals.[8,9,10,11]

Saccharomyces cerevisae ascomycetous yeast mainly used in the brewing and baking industries, can colonize the human respiratory, urinary and gastrointestinal tract. Infections due to *S. cerevisae* have been reported in patients with underlying chronic diseases and immunosuppression and the yeast infection can present as pneumonia, fungemia, peritonitis, endocarditis, vaginitis, oropharyngeal, urinary tract and skin infections.[2,12]

The difficulty in the isolation and cultural identification of most fungi are obstacles in their diagnosis due to similarities in their colonial and morphological appearances.[13] Consequently, genomic DNA extraction and sequencing technologies such as BLAST are used in their definitive and confirmatory identification. Fungal isolates can be characterized by sequencing the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) with universal primers, ITS-1 and ITS-4, to amplify the ITS target region.[14,15]

Cases of opportunistic pneumoaspergillosis occurring alone, and in combination with other infections excluding *S. cerevisae*, have been recorded even in developing countries like Nigeria, in those immunosuppressed as a result of HIV or AIDS.[5,16,17]

MATERIALS AND METHODS

Study area

The Jalingo area of Taraba State, Nigeria was selected as the study area. According to 2006 census record of the National Population Commission, the State has population figures of 2,300,736 people while Jalingo has an estimated population of 139,845 people with over eighty ethnic groups. Agrarian in nature, farming is the predominant occupation in the city while other occupations complement its economic activities.[18]

Ethical clearance and study population

This cross-sectional hospital-based study was approved by the Institutional Review Board of the Department of Microbiology, Federal University Wukari. Ethical clearance was sought for, and obtained from the Ethical Committee of the Federal Medical Centre (FMC), Jalingo.

Inclusion criteria were HIV-infected out-patients of both sexes with or without cough symptoms, who consented to the study, were above the age of 10 years and who were on current anti-retroviral therapy while the exclusion criteria were out-patients who did not meet all the conditions in the inclusion criteria.

Phlegm collection

One hundred out-patients of the FMC, Jalingo, Taraba State, were each given a dry, clean, leak-proof 20ml sample bottle labelled with identification numbers to produce sputum which was immediately collected. The sex and age of each patient was appended on the bottle label and the sputum macroscopically examined.

Culture, isolation and identification of fungal colonies

Subsequently, an inoculum of sputum was streak platted on sabouraud dextrose agar (SDA) using an inoculating loop. The SDA culture plate was incubated at 25^0C and examined after three days of growth till an extended period of 7 days. Isolated microbial colonies were picked off and aseptically transferred to sterile SDA media to obtain pure colonies that were eventually examined macroscopically and microscopically. Pure colonies were characterized based on their colonial morphology: form, margin, shape, colour, topology, opacity, surface texture and diffusible pigments while the wet, and lactophenol cotton blue (LCB) mounts were used to identify hyphal morphology including the spores.[19,20] Data obtained was expressed as percentages.

DNA extraction and amplification

The basic local alignment search tool (BLAST), was used to examine the DNA and protein sequences two distinct genera of these isolates, infer functional and evolutionary relationships between sequences and help to identify members of gene families and species. Genomic DNA was extracted from each pure fungal culture using the Quick-DNA™ Miniprep Plus kit (Zymo Research, Catalogue No. D4068). The OneTag® Quick-load® 2x Mater Mix (NEB, Catalogue No. M0486) was used to amplify the COI target with ITS primers. The target of ITS-1 and ITS-4 was ITS rDNA while the sequence (5` to 3`) of ITS-1 and ITS-4 was TCCGTAGGTG AACCTGCGG and TCCTCCGCTTATTGATATGC respectively.[14,15]

DNA sequencing and purification

The PCR products were run on a gel and enzymatically cleaned up using the EXOSAP method. The extracted fragments were sequenced in the forward and reverse direction using (Nimagen, BrilliantDye™ Terminator Cycle, Sequencing Kit V3.1, BRD3-100/1000) and purifed (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit, Catalogue No. D4050). The purified fragments were analysed on the AB1 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction and every fungal sample. Thereafter, BioEdit Sequence Alignment Editor Version 7.2.5 was used to analyse the ab1 files generated by the AB1 3500XL Genetic Analyzer. The results obtained by a BLAST search from the national center for bioinformatics information (NCBI) were used to confirm the identities of these strains.[14,15]

Statistical analyses

The frequency, percentage, rate, or prevalence of occurrence was expressed as a percentage.

RESULTS

Table I shows the colony (morphological) and microscopic characteristics of *Aspergillus* and *Penicillium* sp. recovered from all sample cultures. There were observational variances in their cultural and cellular appearances on SDA plates and the microscope respectively.

Table- I: Morphological characteristics of fungi

S/N	Colonial characteristics	Reverse surface reaction	Microscopic appearance	Probable organism
1.	Wooly at first white to yellow, then turning to dark brown black	White to yellow	Double cover, entire vesicle form "radiate" head	*Aspergillus* sp.
2.	Velvety, yellow to green	Golden to red brown	Single and double cover, entire vesicle point out in all directions	*Aspergillus* sp.
3.	Blue-green pigmentation with suede-like surface consisting of a dense felt of conidiophores	Pale yellow	A single series of phialides, rounded and rarely conidia	*Aspergillus* sp.
4.	Greenish-blue with whitish edge	Yellow to brown	Septate hypha, columnar conidial head	*Aspergillus* sp.
5.	Tan to brown	Brown	Globose conidia with varying sizes that are slightly roughed	*Aspergillus* sp.
6.	White colour	Pale yellow	Singly phialide, branched metulae Singly phialide, branched metulae	*Penicillium* sp.

Table II represents the prevalence and distribution of fungi. The overall prevalence of fungi infection was 79.0. The sex- and age-related distribution profile of fungi shows that 80.0% males and 78.3% females were infected. In both sexes, fungi were isolated in 92.9% of patients that were between 30 and 39 years and 64.7% in those between 15 and 29 years.

Table- II: Prevalence and distribution of fungi

Age group (years)	Number examined			Number infected		
	Male	Female	Total	Male	Female	Total
10-19	2(5.00)	5(8.30)	7(7.00)	1(3.50)	2(2.80)	3(42.9)
20-29	4(10.0)	6(10.0)	10(10.0)	3(7.50)	5(8.30)	8(80.0)
30-39	12(30.0)	16(26.7)	28(28.0)	10(23.3)	16(28.0)	26(92.9)
40-49	9(22.5)	13(21.6)	22(22.0)	7(17.1)	9(15.2)	16(72.7)
50-59	5(12.5)	9(15.0)	14(14.0)	4(11.2)	7(10.9)	11(78.6)
60-69	8(20.0)	11(18.3)	19(19.0)	7(16.6)	8(13.8)	15(78.9)
Total	40(40.0)	60(60.0)	100(100)	32(80.0)	47(78.3)	79(79.0)

Table III states the molecular BLAST of pure fungal isolates; here the molecular identification of the two queried *Aspergillus* species that were culturally alike using the BLAST technology showed that the similarities between them and biological sequences in the NCBI database, was 99.7%. Ultimately, their identity was confirmed as *Aspergillus flavus* and *Saccharomyces cerevisiae.*

Table- III: Molecular BLAST of pure fungal isolates

S/N	Culturally characterized pure isolate	GenBank Accession Number	Percentage ID (%)	Predicted fungi
1.	*Aspergillus flavus*	XR- 002086443.1	99.7	*A. flavus*
2.	*Aspergillus niger*	NR- 132207.1	99.7	*S. cerevisiae*

Table IV contains the prevalence and distribution of fungal genera. In both sexes, the recovery rates of *A. flavus* and *S. cerevisiae* was 48.1% and 24.1% respectively. In all cases of fungal infection, 47 (78.3%) females were infected, where males were 32 (80.0%) more. *Penicillium* species were also 22 (27.8%) and *A. flavus* were 38 (48.1%).

Table- IV: Prevalence and distribution of fungal genera

Sex	Number examined	Number infected	Number positive			Total
			A. flavus	S. cerevisiae	Penicillium sp.	
Male	40	32	17(21.3)	6(7.50)	9(11.3)	32(80.0)
Female	60	47	21(26.8)	13(16.6)	13(16.6)	47(78.3)
Total	100	79	38(48.1)	19(24.1)	22(27.8)	79(79.0)

Table V shows that the prevalence of non-concomitant *A. flavus* and *S. cerevisiae* in 57% male and female patients in six (6) age categories. There was no established pattern of infection across ages and sexes. However, the overall number of infected females was 61.4%) and infected males was 38.6%.

Table- V: Prevalence and distribution of Aspergillus flavus and Saccharomyces cerevisiae

Age group (years)	Number examined (n =100)			Number infected (n=57)			Distribution of A. flavus (n = 38) and S. cerevisiae (n = 19)						Total
	Male	Female	Total	Male	Female	Total	Male			Female			
							A. flavus	S. cerevisiae	Total	A. flavus	S. cerevisiae	Total	
10-19	2(5.00)	5(8.30)	7(7.00)	0(0.00)	1(1.40)	1(14.3)	0(0.00)	0(0.00)	0(0.00)	1(1.00)	0(0.0)	1(57.0)	1(14.3)
20-29	4(10.0)	6(10.0)	10(10.0)	1(2.50)	4(6.70)	5(50.0)	1(5.00)	0(0.00)	1(11.4)	2(2.50)	2(5.5)	4(45.6)	5(50.0)
30-39	12(30.0)	16(26.7)	28(28.0)	5(11.7)	9(15.8)	14(50.0)	3(8.40)	2(5.60)	5(20.4)	5(7.70)	4(6.2)	9(36.6)	14(50.0)
40-49	9(22.5)	13(21.6)	22(22.0)	6(14.7)	7(11.8)	13(59.1)	4(8.70)	2(4.30)	6(26.3)	5(9.30)	2(3.7)	7(30.7)	13(59.1)
50-59	5(12.5)	9(15.0)	14(14.0)	3(8.40)	6(9.30)	9(64.3)	3(9.00)	0(0.00)	3(19.0)	4(6.00)	2(3.0)	6(38.0)	9(64.3)
60-69	8(20.0)	11(18.3)	19(19.0)	7(16.6)	8(13.8)	15(78.9)	4(8.60)	3(6.40)	7(26.6)	6(11.3)	2(3.8)	8(30.4)	15(78.9)
Total	40(40.0)	60(60.0)	100(100)	22(55.0)	35(58.3)	57(57.0)	15(38.9)	7(18.1)	22(38.6)	23(37.5)	12(19.5)	35(61.4)	57(57.0)

Figures in parentheses represent percentages

DISCUSSION

Aspergillosis has been associated with significant morbidity and mortality among immune-compromised patients.[21,23] This present study provides current microbiological evidence that pulmonary *Aspergillus* infection is more common with HIV infected persons undergoing treatment than other fungal infections. However, this study highlighting the high recovery of *S. cerevisae* should elicit concerns in the management of both diseases as this is the first record of its occurrence in Nigeria in those immunosuppressed due to HIV or AIDS and who are currently and regularly receiving antiretroviral treatment.

The results herein interestingly show that *A. flavus* and *S. cerevisae* infections are not age- or sex dependent. The implication of this is that transmission can equally occur in any person irrespective of his or her age.[16] More worrisome is the fact that the prevalence of *A. flavus* obtained in this current study was higher than those of Ogba *et al.*[23] and Nasir *et al.*[24] who respectively recorded occurrences of 47.1% and 12.7% in Southern and Northern Nigeria, as well as those of Kaur *et al.*[16] and Prakash *et al.*[25] who reported a prevalence of 16.9% and 16.5% respectively in India. However, these discrepancies might be as a result of the differences in the sample size, defining inclusion criteria and methodologies employed.

Several genetic techniques have been used to classify different *Aspergillus* species, namely the ITS (internal transcribed spacer) region, the aflatoxin gene cluster, and random amplification of polymorphic DNA (RAPD).[14,23] In this current study, the genomic DNA containing 16srDNA specific primers that were helpful in amplifying medically important fungi were used. All the fungal isolates tested appeared very heterogenous. This allowed the exclusion of a common source of infection. The

non-relatedness of *A. niger* sequential isolates could suggest a pattern of re-infection rather than relapse unlike those of *A. flavus*.

Following the recommendation of the World Health Organization.[20] that diagnostic services for confirmation of their causative role in AIDS and mortality be strengthened, it is therefore necessary to identify and carefully monitor patients with high risk factors for developing AIDS and to initiate diagnostic procedures as soon as possible in these patients. The results of the study showed that the BLAST method is diagnostically significant in the characterization of closely related *Aspergillus* and *Saccharomyces* species found in the respiratory tract of humans infected with HIV and whose presence may account for the morbidities observed even while they are currently receiving anti-retrovirals. Considering that as at the end of December 2022, the Joint United Nations Programme on HIV/AIDS (UNAIDS) documented the fact that 29.8 million people were accessing ART up from 7.7 million in 2010.[26]

CONCLUSIONS

Accurate and timely diagnoses of these fungal infections are likely to positively affect HIV treatment outcomes as well as the epidemiological pattern of HIV/ AIDS. In this regard, all HIV-positive individuals, regardless of whether they have cough symptoms or not, should be appropriately tested early for respiratory tract fungal pathogens. Success can be increased by collaborative groups of expertise in diagnostics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Original Article

Clinicobiochemical Parameters of Cytomegalovirus IgM and IgG positive Biliary Atresia and their Relation with Serological Titer in Infants

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Abstract

Cytomegalovirus (CMV) associated Biliary atresia (BA) is one of the clinical classification of Biliary atresia (BA). There is a hypothesis that CMV IgM positive BA is a clinically different entity and prognosis is poor. The aim of this study was to evaluate the clinical and biochemical parameters of CMV IgM and CMV IgG positive BA in one to six month old infants and their relation with serological titer. This cross-sectional study was carried out from January 2019 to June 2022 in the department of Pediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. A total of 48 infants were included in this study as study subject who were diagnosed as biliary atresia with positive CMV IgM & CMV IgG. After taking written informed consents data were taken from parents or legal guardians by using a structured questionnaire. Data were analyzed by statistical package for social sciences (SPSS), version- 23. Majority of the cases (58.3%) were in 2-4 month of age group, 70.8% were male and male-female ratio was almost 2.5:1. Regarding birth history most of the infants (95.8%) were term baby and two-third (66.7%) of them was appropriate for gestational age (AGA). This study observed the onset of jaundice among the infants, here 60.4% of them detected jaundice jaundice within 7 days, 22.9% within 7-14 days and 16.7% after 14 days. About three fourth (73%) of infants presented with intermittent pale stool and more than one-fourth (27%) had persistent pales tool. One fourth 12(25%) patient had features of coagulopathy. The mean of total bilirubin, direct bilirubin, alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT) were 11.89±4.0, 8.27±3.42, 162.67±103.09 and 669.46±543.57 respectively; The mean of the titers of CMV IgM and CMV IgG were 50.84±33.09 and 82.40±53.79 respectively. The prolong international normalised ratio (INR) was in 30.8% of infants and mean INR was 2.44±2.54. Study finds that CMV IgM titer level was correlated with total bilirubin (r=-0.256; p<.05) and direct bilirubin (r=-0.365; p<.05); where CMV IgG titer level was correlated with age (r=-0.362; p<.05) and INR (r=0.271; p<.05). It may be concluded as increase in age would increase titer of IgG and increase in titer of IgG is associated with increase coagulopathy. Elevated levels of CMV IgM titer correlated with bilirubin level or cholestasis.

Keywords: *Biliary atresia, cytomegalovirus IgM, cytomegalovirus IgG, clinicobiochemical parameters, antibody titer.*

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INTRODUCTION

Biliary atresia (BA) is a destructive, obliterative cholangiopathy of the newborn characterized by a variable degree of obliteration of both intrahepatic and extrahepatic bile ducts eventually causing severe cholestasis progressing to biliary cirrhosis.[1]

Japanese Association of Pediatric Surgeons classified biliary atresia (BA) into three types based on the extrahepatic bile duct obstruction. Clinically, biliary atresia (BA) is classified as isolated, typical biliary atresia (80%), cystic biliary atresia (5-10%), Biliary atresia-splenic malformation syndrome (BASM: 5-15%), cytomegalovirus (CMV) associated biliary atresia (5-10%).[2] Clinical course & prognosis of these four type of biliary atresia (BA) may vary whether these different type have different causes is

unknown.[2] In this study our concern is about cytomegalovirus (CMV) associated biliary atresia (BA) on the basis of cytomegalovirus IgM, cytomegalovirus IgG positivity . It is believed that biliary atresia (BA) is disorder of multifactorial etiology. Infectious etiology, especially viral infection proposed as precipitating factor of biliary atresia (BA) by several studies. Infection targeting the bile duct induced an auto-immune response leads to chronic fibrosclerosing injury.[3,4] Among several viruses cytomegalovirus (CMV) considered to be the most common virus related to biliary atresia (BA).[5, 6] At first serological evidence of cytomegalovirus (CMV) exposure support this speculation, later polymerase chain reaction (PCR) methods used for the detection of cytomegalovirus (CMV).[7] However, direct virus isolation or inclusion bodies could not be possible yet to conclude the hypothesis.[8] 30–40% of infants with biliary atresia (BA) showed Serological evidence of cytomegalovirus (CMV) infection in study from different countries.[9,10,11] Cytomegalovirus (CMV) -specific liver Tell response was also found in more than 50 % infant with biliary atresia (BA) with positive correlation of this laboratory finding with plasma cytomegalovirus IgM levels.[12] Recently a meta-analysis showed higher prevalence (25.4%) of cytomegalovirus (CMV) infection in infants with biliary atresia (BA). Moreover, the prevalence rate of cytomegalovirus (CMV) infection is higher in Asia than in Europe & this high prevalence have relation with lower socioeconomic status.[13] Zani A et al conducted a study in 2015, showed cytomegalovirus (CMV) associated biliary atresia (BA) infants clinically different from non cytomegalovirus (CMV) associated biliary atresia (BA), relatively older at Kasai portoenterostomy (KPE), more severe biochemical derangement in cytomegalovirus (CMV) associated biliary atresia (BA) infants.[14] Also recently a trial antiviral therapy showed better outcome in cytomegalovirus (CMV) associated biliary atresia (BA) . The aim of this study was to evaluate clinical, biochemical parameters and serological titers of infants with biliary atresia (BA) & to determine correlation between cytomegalovirus IgM, cytomegalovirus IgG titer level and different clinical & biochemical parameters in cytomegalovirus (CMV) associated biliary atresia(BA) whether any influence on severity & outcome of the disease.

MATERIALS AND METHODS

This cross sectional study was conducted in the Department of Paediatric Gastroenterology and Nutrition, BSMMU during the period from January 2019 to June 2022. A total of 48 infants were included in this study as study population. Considering selection criteria infants of diagnosed biliary atresia with cytomegalovirus IgM and cytomegalovirus IgG positive were selected from the the Department of Paediatric Gastroenterology and Nutrition study subjects. Parents or legal guardians of infants were considered as respondents. A structured questionnaire and a data sheet were designed with a view to collect data from the respondents. Then detail clinical history, physical examination findings and investigation reports were recorded in the preformed data sheet. With all aseptic precautions venous blood was drawn for laboratory work up. Cytomegalovirus IgM and cytomegalovirus IgG antibodies were measured using Chemiluminescence ELISA technique at Virology Department of BSMMU. A cytomegalovirus IgM positive was defined as anti-CMV IgM > = 22AU/ml and a positive cytomegalovirus IgG was defined as anti-CMVIgG > = 14 AU/ml, in accordance with manufacturer's instructions. Percutaneous liver biopsy were performed using Tru-cut biopsy needle by the same expert in all cases. Before liver biopsy we ensured normal vital parameters, normal platelet counts, normal coagulation profile and no cystic lesion in the liver in Ultra-sonogram (USG). Informed written consent was taken from each parent or legal guardian.

Statistical Analysis: Statistical analyses were performed using the Statistical Program for Social Sciences (SPSS) software (version 23.0; SPSS, Inc.). Categorical variables were presented as frequencies and percentages in tables and graphs; continuous variables were expressed as means and standard deviation. Correlations were assessed with the Pearson's correlation coefficient. A p-value of less than 0.05 was considered statistically significant. The approval of the research protocol was obtained from the Departmental Review Board of BSMMU.

RESULTS

Total 48 histologically confirmed BA infants associated with CM-IgM and CM-IgG positive cases were studied. Mean age of infants was 3.37±1.45 months, where 34 (70.8 %) were male and male-female ratio was almost 2.5:1.

Table I contains the distribution of clinical features of cytomegalovirus IgM and cytomegalovirus IgG positive BA. Among the cases 28 (58.3%) were in age group 2 to 3

months, 4 (8.4%) were <2 -3 months, 16 (33.3%) were >3 months at the date of admission. Among 48 cases. Regarding birth history 31 (64.6%) infants were delivered by lower uterine caesarian section (LUCS) and rest 17 (35.4%) were normal delivery. Normal birth weight with appropriate gestational age (AGA) were found in 32 (66.7%) infants and 16 (33.3%) had low birth weight. Among the infants 46 (95.8%) were term and rest 2(4.2%) were preterm. Infants mean age of appearance of jaundice was 7.67±10.98 days, where jaundice was traced before 7 days in 29 (60.4%) cases, within 7-14 days in 11 (22.9%) and after 14 days in 8 (16.7%) cases. Presence of intermittent pale stool was detected in 35 (73%) cases and the remaining had persistent pale stool 13 (27%). History of maternal fever and rash were detected in 4 (8.3%) cases. Feature of coagulopathy was present in 12 (25%) cases, where liver was palpable in 40 (83.3%) and spleen was palpable in 24 (50%) cases.

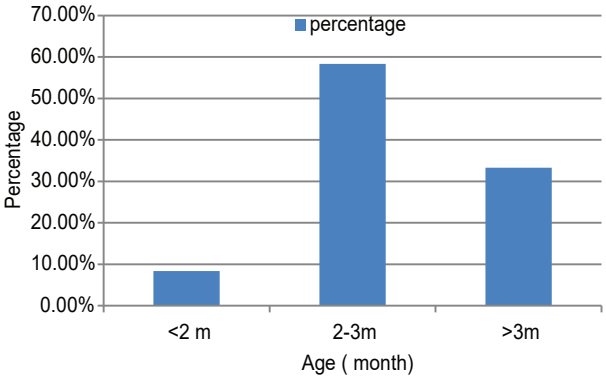

Figure- 1: Age distribution of studied subjects (n=48)

Figure 1 represents the age distribution of studied subjects, here 58.3% infants were in 2 to 3 months of age group, 8.4% were <2 months and 33.3% were >3 months of age at the time of admission.

Table- I: Clinical Features of CMV IgM & IgG positive Biliary atresia (n=48)

Clinical variables	Observations	Frequency	Percentage
Age group	<2 month	4	8.4
	2-3 month	28	58.3
	>3month	16	33.3
Sex	Male	34	70.8
	Female	14	29.2
Birth weight	Appropriate for gestational age (AGA)	32	66.7
	Low birth weight(LBW)	16	33.3
Birth history	Normal vaginal delivery (NVD)	17	35.4
	Lower uterine caesarian section(LUCS)	31	64.6
	Term	46	95.8
	Preterm	2	4.2
Jaundice onset	< 7 days	29	60.4
	7-14 days	11	22.9
	>14 days	8	16.7
Pale stool	Persistent	13	27.0
	Intermittent	35	73.0
Feature of coagulopathy		12	25
H/o maternal fever & or rash		4	8.3
Liver (palpable)		40	83.3
Spleen (palpable)		24	50.0
Total		48	100

Table II shows hematological and biochemical parameters of BA with CMV IgM and CMV IgG positive cases at the time of diagnosis. Mean hemoglobin level, mean ESR, mean platelet count and mean total WBC count were 9.4 ±1.53, 28.2±18.81, 305×10^9/L and 15.16×10^3/L respectively. Mean total and direct bilirubin was 11.89±4.07 and 8.27±3.42 respectively. Mean alanine transaminase (ALT) was 162.67±103.09, mean Gamma-glutamyl transpeptidase (GGT) was 669.46±543 and mean INR was 2.44±2.54. Mean CMV IgM and CMV IgG titer were 50.84±33.09 and 82.40±53.79 respectively.

Table- II: Hematological & biochemical parameters subjects with CMV IgM and CMV IgG positive Biliary atresia (n=48)

Hematological & Biochemical parameters	Mean	±*SD*
Hemoglobin (gm/dl)	9.4	1.53
ESR	28.2	18.81
Total count WBC[c] (×10^3 /L)	15.16	7.96
Platelet[c] (×10^9/L)	305	350
Total bilirubin (mg/dl	11.89	4.07
Direct bilirubin (mg/dl)	8.27	3.42
Alanine transaminase (IU/L	162.67	103.09
Gamma-glutamyl transpeptidase (IU/L)	669.46	543.57
International normalised ratio (INR)	2.44	2.54
Cytomegalovirus IgM	50.84	33.09
Cytomegalovirus IgG	82.40	53.79

Table III states distribution of patient by INR, level <1.2 in 24 (50%), 1.2-1.5 in 9 (19.2%) and >1.5 in 15 (30.8%) cases.

Table- III: Distribution of patient by International normalised ratio (INR) level (n=48)

INR	Frequency	Percentage
<1.2	24	50.0
1.2-1.5	9	19.2
>1.5	15	30.8
Total	48	100

Table IV shows the correlation between serum level of CMV IgM, CMV IgG titers including clinical and biochemical parameters. Serum CMV IgM titers were correlated with total bilirubin (r=-0.457; p=0.028), and direct bilirubin (r = -0.488; p=0.018). Serum CMV IgG titers were correlated with age (r = -0.530; p=0.001) and INR (r=0.593; p=0.005). Alanine transaminase (ALT) and Gammaglutamyl transpeptidase (GGT) were not significant correlation with CMV IgM, CMV IgG titers.

Table- IV: Correlation of serum CMV IgM and IgG titer with age; various biochemical parameters of CMV IgM and IgG positive titer with Biliary Atresia (n=48)

Clinical & Biochemical parameters	Cytomegalovirus IgM titer		Cytomegalovirus IgG titer	
	r-value	p-value	r-value	p-value
Age	-.301	0.153	0.5300	0.011
Total bilirubin	.457	0.028	.111	0.622
Direct Bilirubin	.488	0.018	.142	0.527
Alanine transaminase (ALT)	.096	0.656	.183	0.415
Gammaglutamyl transpeptidase (GGT)	0.057	0.806	-.092	0.698
International normalised ratio	-.279	0.198	.593	0.005

Correlations were assessed using Pearson's correlation coefficient.

DISCUSSION

Biliary atresia (BA) is a rare disease, but incidence of biliary atresia (BA) is 25.8% in our country.[15] Prevalence of cytomegalovirus (CMV) infection is higher (25.4%) in biliary atresia (BA) infant than congenital cytomegalovirus (CMV) infection in general population.[13] As a tertiary care center there is a group of biliary atresia (BA) patient in our institute who are CMV IgM & IgG antibody positive & they are sufficient in number for the study purpose. Still now there is controversy whether cytomegalovirus (CMV) infection has a role in the pathogenesis of biliary atresia or isolated pathology as clinical evidence is poor.[16] so it is reasonable to study biliary atresia (BA) associated with cytomegalovirus (CMV) from a different point of view. Mean age of the studied cytomegalovirus IgM & IgG positive biliary atresia (BA) patients at admission was 3.37±1.45 month. In a previous study conducted at the same center showed mean age was 3.3 months in biliary atresia (BA) infant.[17] Another study mention

cytomegalovirus IgM positive biliary atresia (BA) patient were older 70 days at Kasai Porto-enterostomy (KPE) than cytomegalovirus IgM negative biliary atresia (BA).[14] Biliary atresia is common in female infant as well as cytomegalovirus IgM positive biliary atresia(BA) is also reported to be common in female infants.[14,18] Biliary atresia (BA) is common (61.6%) in male infant in previous study at the same center conducted in 2005.[17] Now there is also male predominance 34(70.8%) in cytomegalovirus IgM & IgG positive Biliary atresia. There may be symptom of maternal Primary cytomegalovirus (CMV) infection during pregnancy period including fever, headache, malaise, pharyngitis, hepatosplenomegaly, lymphadenopathy, arthralgias, and rash causing congenital cytomegalovirus (CMV) infection. In our study history of maternal fever & rash was present in only 4(8.3%) indicating that there may be perinatal CMV infection rather than congenital infection**.** However, this symptom also may present in Epstein Barr virus (EBV) infection.[19] Infant with congenital CMV infection present with symptoms of Jaundice, petechiae, chorioretinitis, and cataracts hepatosplenomegaly intracranial calcifications, microcephaly, ventriculomegaly intrauterine growth restriction, pericardial effusion, premature or small for gestational age, hearing loss. Our patient had no feature of congenital CMV infection except jaundice & hepatosplenomegaly. Liver is palpable in 40(83.3%) child which is common feature of biliary atresia & CMV infection. Spleen was palpable in 24 (50%) child may be as part of CMV infection because there is no evidence of accessory spleen or double spleen evident by ultrasonography & no feature of biliary cirrhosis. Hearing loss is the most common sequelae associated with congenital CMV infection. Hearing assessment done in all of our patient reporting no hearing impairment. This finding also excludes congenital CMV infection. 32(66.7%) of our child had appropriate for gestational age (AGA) & 16(33.3%) had low birth weight, 46(95.8%) were term, 2(4.2%) were preterm baby. Congenital CMV infection could be excluded by doing CMV DNA or IgM within 3 weeks of delivery, which was not possible for our study due to late presentation.[19] Biliary atresia associated with positive CMV IgM differs from other types of biliary atresia by late onset clinical manifestations. The patient of CMV IgM positive BA appears healthy during birth, but obstructive cholestasis is developed after the second week of life & gradually progress over time.[20] In our study mean age of onset of jaundice was 7.67±10.98 days. Jaundice started to appear before 7 days in 29 (60.4%) cases, within 7-14 days in 11(22.9%) cases & after7 days in 8(16.7%) cases. Feature of coagulopathy was present in 12(25%) cases. In a previous study CMV IgM positive group BA had pale stool in 47.4% cases, pigmented stool 52.6% cases. Presence of pigmented stool was more common (35, 73%), the remaining had persistent pale stool (13, 27%) in this study. As for liver function, the median levels of alanine transaminase (ALT), total bilirubin, direct bilirubin, Gamma-glutamyl transpeptidase (GGT) were 140, 9.5, 6.08, and 377 respectively.[21] In Our CMV IgM & IgG positive BA patient mean total & direct bilirubin was 11.89±4.07, 8.27±3.42 respectively, mean alanine transaminase (ALT), was162.67±103.09, mean Gamma-glutamyl transpeptidase (GGT) was higher 669.46±543, mean INR was 2.44±2.54. Different study uses different test method to detect presence of CMV infection like serum CMV IgG, CMV IgM, and CMV-DNA, CMV-pp65. We used CMV IgG & CMV IgM, because urinary CMV DNA was not available during study period in our institute. However if number of virus less than the limit of PCR sensitivity, CMV infection cannot be ruled out by CMV DNA. Moreover in case of persistent infection intermittent shedding of virus occur.[7] Mean CMV IgM, CMV IgG titer were 50.84±33.09, 82.40±53.79 respectively in our study There is positive clinical correlation between age & serum level of CMV IgG (r=-0.530; p=0.001) & statistically significant indicating increase in age would increase titer of IgG. Serum CMV IgG titers were also positively correlated with INR (r=0.593; p=0.005) levels. So increase titer level associated with increased INR level or coagulopathy. Serum CMV IgM titers were positively correlated with total bilirubin(r=-0.457; p=0.028) & direct bilirubin (r=-0.488; p=0.018). Increase in serum CMV IgM titers would increase total & direct bilirubin level. Previous studies suggested that newborn infants with congenital Cytomegalovirus infection could be identified by CMV IgM reactivity and elevated levels of CMV IgM correlated with the disease severity. Manifestations of CMV infection reflect the level of virus replication as well as the end-organ involvement.[22]

CONCLUSIONS

This study concluded that about three fourth of CMV IgM and IgG positive BA patient presented with pigmented or intermittent pale stool, one fourth patient had feature of coagulopathy. Most of the infants were term baby and half of them had splenomegaly. Study fiends that an increase in age would increase titer of IgG and increase in titer

associated with increase coagulopathy. Elevated levels of CMV IgM titer correlated with bilirubin level or cholestasis. However, multi-center based study with large sample size may need to reach a consensus.

LIMITATION

It was a single center study with small sample size. Though real - time PCR is more sensitive and specific for detection of primary CMV infection than CMV IgM and CMV IgG.

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Original Article

Diagnosis and Prognosis of Neonatal Septic Arthritis from It's Early Clinical Characteristics: Experience from a Tertiary Care Hospital

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Abstract

Neonatal septic arthritis (NSA) is an unusual illness, but fatal clinical condition and must be diagnosed and treated properly in order to save the neonate's joints and life. High rate of morbidity and mortality may result from NSA if left untreated. Majority of NSA cases in developing countries present themselves late at the health care facilities, making the diagnosis difficult and sometimes overlooked by health care providers. Although septic arthritis has already been investigated but cases of NSA from developing countries are under reported. The goal of the study was to better understand the clinical and microbiological characteristics of neonates with septic arthritis as well as their prognosis of treatment. This prospective observational study was conducted in Neonatal Intensive Care Unit (NICU) of Department of Neonatology and Out Patient Department (OPD) of Orthopaedics, Banga bandhu Sheikh Mujib Medical University (BSMMU). Total 30 neonate presented with acute septic arthritis were included in this study. Data were collected by reviewing record from NICU and disease course, investigation profiles, treatment record of neonates were noted as recorded during the time of hospital stay or attending in OPD. For the assessment of prognosis clinical and radiological findings of all cases were followed-up for 12 months of age. Collected data of neonates with septic arthritis from record review were processed and analyzed by Statistical Package for the Social Sciences (SPSS) and result of analyzed, finally data were presented as in tables and graphs. This study finds that nearly two-third (63%) of the neonates were male and knee joint was most frequently involved, accounting more than two-third (67%) of all single joint involvements. All of the cases were anemic and had high C-reactive protein (CRP) level. Joint aspirate samples culture showed that the most prevalent (41%) organism was Klebsiella, followed by Acenotobacter and Seratia. Gram-negative bacteria predominated (91%). Most of the patient (76%) had favorable prognosis after treatment. Clinical evaluation and start of intravenous antibiotics should be given priority in suspected cases. Prompt surgical intervention and consistent follow-up in a tertiary hospital are necessary for the best results.

Keywords: *Neonate; Septic arthritis, Arthrotomy, outcome.*

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INTRODUCTION

Septic arthritis is one of the most incapacitating illnesses for people of all ages.Infants and children are more likely to develop septic arthritis due to innate impairments and limits in defensive mechanisms. Despite being uncommon in newborn sepsis, bone and joint infections account for a considerable proportion of cases in developing countries.[1] "The incidence of neonatal septic arthritis is approximately 0.3 per 1000 live births worldwide, while it has been estimated that the rate in India is 0.6 per 1000 live births."[2] Due to the lack of initial signs and symptoms, neonatal septic arthritis usually goes untreated, yet it can have serious consequences, including the newborn's death. The most prevalent organism in culture is Staphylococcus aureus, However, other species such as Klebsiella pneumomiae, Group B streptococci, Escherichia coli, Enterobacter sp., Kingella kingae, and Candida sp have been isolated in culture.[3] Secondary hematogenous seeding of infectious organisms is a common mechanism of infection. The increased vascular supply and lack of synovial basement membrane in newborn infant joints predispose to this condition.[4] Due to the immaturity of a newborn's immune system, the illness has a high potential to spread swiftly and result in a number of terrible outcomes, including as sepsis, osteomyelitis, meningitis, the creation of abscesses in tissue gaps, and urinary tract infections. Such individuals may experience long-term

morbidity from septic arthritis due to restrictions on normal joint motion, as well as the deterioration of articular cartilage and ossification sites.[5] It is an orthopedic emergency, and if it is not treated quickly, there could be major morbidity and fatality. The diagnosis of septic arthritis is extremely important and difficult. Joint injury, subluxation, dislocation, and other catastrophic consequences might result from delayed diagnosis and treatment. Age, the type of organisms, their virulence, patterns of resistance, and the length of the infection all play a role in the outcome. Compared to wealthy nations, septic arthritis is more prevalent in underdeveloped nations.[6] Early detection and the use of appropriate care result in favorable outcomes. Delays in treatment worsen the prospects for recovery.[7] Neonatal septic arthritis is underreported in developing countries despite being extensively researched in paediatric age grou.[8,9]

There is limited literature evidence exists from Bangladesh on neonatal septic arthritis. So, we tried to 6+investigate along with the outcomes of long-term follow-up, the clinical and bacteriological profiles.

MATERIALS AND METHODS

From January 2021 to December 2022, this prospective observational study was carried out in the Department of Neonatology and OPD of the Department of Orthopaedic Surgery at the Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka, Bangladesh. This study covered all term and preterm neonates who displayed the signs of septic arthritis. This study excluded neonates with congenital bone abnormalities and other congenital defects. A case was considered to be neonatal septic arthritis if presented with swelling of joints or met Morrey's diagnostic criteria of septic arthritis. "Morrey's criteria was satisfied with at least 2 of major criteria, namely, pus aspirated from the joint, marked elevation of erythrocyte sedimentation rate, specific roentgenographic changes in the involved site, and at least 5 of the minor criteria such as fever greater than 38.3°C, pain (localized to the joint) made worse by gentle passive motion, swelling of the involved joint, systemic symptoms of lethargy, malaise, irritability, no other demonstrable pathological process, satisfactory response to antibiotic therapy, and supportive evidence of ultrasound showing joint fluid collection"[10]. Informed consent was obtained from the parent of all the neonates. Those neonates who were admitted in NICU of BSMMU, clinical profile were collected in pre-designed data sheet from medical records. Birth weight, comorbidities, gestational age, success of surgical and antibiotic therapy, and organisms cultured had all been noted. After initial treatment follow up and further management given at OPD of Department of Orthopaedic surgery in BSMMU. Those who were directly attended in OPD of Department of Orthopedic surgery, BSMMU after initial treatment taken from outside details history, investigation profile and management history were collected from previous medical records.

Following discharge, all cases were followed up with three times a month until the child was 12 months old. Clinical evaluations were performed on all patients, including joint examination, range of motion (ROM), and pain level. Every appointment also included a check for mobility restriction, persistent joint deformity, and limb length discrepancy. Plain roentgenographic evaluation of the affected joint was performed every three months, along with a haematological test, and the ultimate outcome was graded after one year. Clinical examination and/or radiological findings such as absence of epiphyseal ossification, presence of a small epiphysis, metaphyseal widening, dislocation, or subluxation noted on follow-up were used to define poor outcomes as limb length discrepancy of more than 1 cm or restricted joint mobility. Institutional review board approval was waived for this study as data were collected from medical records.

Data were analyzed using IBM SPSS software package version 25.0 (IBM Corp, Armonk, New York, USA). The mean, standard deviation (SD), median, and range for numerical variables, and counts and percentages for categorical variables, were used to summarize the data using conventional descriptive statistics.

RESULT

We collected 36 infants with neonatal septic arthritis from actual medical records. Thirty of these 36 newborns were available for follow-up. Out of 30 infants 18 neonate got treatment in NICU of BSMMU and 12 neonate attended OPD for follow up who got initial treatment outside the BSMMU. Two infants died after discharge, while one infant passed away while in the hospital. We monitored the remaining 30 infants, by clinical and radiological examinations, and recorded our findings for a 12-month period.

The follow-up group received an average of 3 visits. 23 (76%) of the 30 newborns had good prognosis. During clinical assessment, we noticed that 7 newborns (24%) had

unsatisfactory clinical outcomes, including length disparity (14%), restricted range of motion (31.4%).Seven infants were found to have poor radiological outcomes, including tiny epiphyses, absence of epiphyseal ossification, dislocations, subluxations, metaphyseal widening, and persistent osteomyelitis. Although one infant's X-ray results was abnormal, but clinically well.

Table I shows tha among the patient's male was most prevalent 19 (63.3%).The median (range) birth weight age was 2.7(1.2-3.4) and gestational age was 37(29-40). Among them 36% was preterm and 40% was low birth weight baby. Age of presentation was 27.6±4.3 days. 70% of infants had comorbidities, and 80% of them had a history of hospitalization. Venous lines were used during the previous hospitalization of 20 (66.6%) newborns.

Table- 1: Baseline characteristics of the enrolled cases (n= 30)

Background characteristics	Neonate n=30 (%)
Male	19 (63.3)
Birth weight(kg)ŧ	2.7 (1.2-3.4)
Low birth weight	12 (40)
Gestational age(weeks)ŧ	37 (29-40)
Preterm	11 (36.6)
Age at presentation(days)*	27.60 (4.3)
Comorbidities	21 (70)
History of previous hospitalization	24 (80)
History of previous I/V medication	20 (66.6)
Exchange transfusion	2 (6.6)
History of previous surgery	2 (6.6)
Mechanical ventilation	3 (10)

* mean±SD,ŧ median(range)

Table II contains that joint swelling, tenderness and excessive cry were found as predominant symptoms. Single joint involvement was found common. Knee joint found more in our study (50%). Low haemoglobin, high CRP were found.76% of blood culture and sixty percent of joint aspirate showed positive. Soft tissue swelling was found as most predominant feature in X ray.

Table- II: Clinical profile of the enrolled neonate (n=30)

Variables	Neonate n=30 (%)
Fever	24 (80)
Joint swelling	28 (93)
Redness of overlying swelling	20 (66.6)
Tenderness	28 (93.3)
Poor feeding	18 (60)
Excessive cry	28 (93.3)
Joint distribution	
Knee	15 (50)
Hip	5 (17)
Multiple	10 (33)
Haematological parameter	
Haemoglobin gm/dl*	9.47 (1.79)
Total leucocyte count WBC/mm3†	13,950 (11000-38,000)
CRP†	73.5 (12-158)
Positive blood culture (n=30)	23 (76.6)
Positive joint aspirate culture (n=20)	12 (60)
Radiological findings	
Soft tissue swelling	29 (96.6)
Increased joint space	12 (40)
Osteomyelitis	8 (26)

*Mean (Standard deviation), †median(range)

Table III represents the distribution of organisms isolated from the culture of blood and aspirated fluid of joint. Here blood cultures were done among 30 cases and 23 (76. 67%) cases were found positive for *klebsiella, Acinetobactor, Seratia, Psedomonas, E colli* and *Candida*; but joint fluid cultures were done among 20 cases and 12 (60%) cases were found positive for all the organisms as blood culture except *E colli*. *Klebsiella* was found in 40% cases from blood culture and in 42% cases from joint fluid culture.

Table-III : Organism isolated from the cultures of blood and joint fluid aspirate

Organism	Blood (n= 23)	Aspirated joint fluid (n= 12)
Klebsiella	7 (30.43%)	5 (41.67%)
Acinetobactor	5 (21.74%)	3 (25.00%)
Seratia	4 (17.39%)	1 (8.33%)
Psedomonas	2 (8.70%)	1 (8.33%)
E colli	3 (13.04%)	0 (0.00%)
Candida	2 (8.70%)	2 (16.67%)

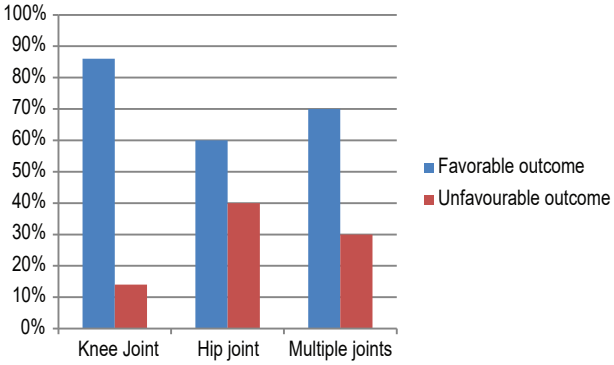

Fig 1: *Prognosis of neonatal septic arthritis according to joint involvement after one year followup (n=30)*

Figure 1 illustrate the prognosis of neonatal septic arthritis according to joint involvement after one year follow up, 86% of neonate who presented with knee joint involvement and 70% of multiple joint involvement had better outcome. Favourable outcome was found in 60% of neonate with hip joint involvement.

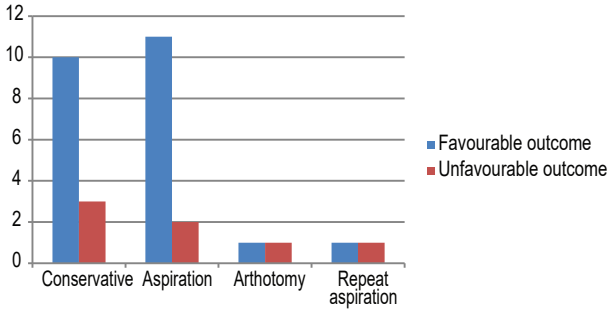

Figure- 2: *Prognosis according to mode of treatment (n=30)*

Figure 2 represent the prognosis of neonatal septic arthritis according to mode of treatment; here 10 (76.9%) and 11 (84%) neonates had satisfactory outcomes who treated conservatively and aspiration respectively. Another 2 neonates who needed repeat aspiration and 50% of them had favourable outcome. Among the neonates who treated with arthotomy, 50% of them found favourable outcomes.

DISCUSSION

Neonates with septic arthritis require special care because of its modest signs and symptoms and severe effects. Septic arthritis in neonates is difficult to diagnose and often missed and leads to seriously disabling sequalae which can be prevented if promptly treated in early stage of infection. We can take preventative measures and determine prognosis with the help of result from our study.

In terms of sex preference, the previously reported data was male-predominant.[1,7] Our cohort had also male sex (63 %) predilection. Female predominance found 2 studies.[11,12] The majority of recent research on newborn septic arthritis focuses on preterm infants.[7,11,12] 64% of the participants in our study were term neonate who were sent to our institution from the outlying areas. Similar kind of findings were observed by Usa et al (63%).[1] Age at presentation was on average 27.6 ±4.3 days in our study.After being exposed to numerous risk factors for blood stream infection either during, prior hospitalization or from the community, babies began to exhibit symptoms after the second week of life.The mean age of onset of symptoms were found 15 days onward in other studies.[1,7,11,13]

Comorbidities and neonatal resuscitation enhance the risk of septic arthritis.[1,13] We observed that the majority (80%) of the newborns were admitted to the NICU for various medical reasons.

The lower limb assumes a flexion, abduction, and external rotation posture with swelling when suffering from newborn septic arthritis.

Pseudoparalysis, or decreased active movement, was present in all the newborns in our series.

In other studies the lower limb, and particularly the hip, is frequently affected.[1,7] According to our findings, the knee joint is most frequently affected (50%). Similar results were discovered in Nishat's study.[11] The higher concentration of organisms at the infection site than in blood can be used to explain the higher yield from joint culture.In our study, blood cultures were positive in 76% of infants and joint fluid cultures were positive in 60% of infants. The joint culture positivity was higher than the

USA (35.7%) but lower than earlier studies by Rudra (78%) and Akash et al. (60%).[1,7,15] Furthermore, in comparison to previous studies, our blood culture positivity was also lower. Long-term antibiotic use while in the hospital can be blamed for these low yields.Joint fluid culture was not done in all cases due the unavailability of expert orthopedic surgeon specially those neonate who treated outside the BSMMU.For this reason exact picture was not represent in our study. Since majority of the patients had taken broad-spectrum antibiotics before being admitted to the hospital. The majority of the organisms in our study were Gram-negative, with Klebsiella being the most prevalent. Gram-negative organisms were mostly documented in the majority of post-millennium studies.[1,7,11,12] However, it has been observed that the rates of fungal septic arthritis are increasing over a period of time due to rampant use of antibiotics .We found 2 cases of candida positive in our study. It has been observed that in the recent past gram negative organisms have replaced gram positive organisms as the most common cause of septic arthritis . Besides, the type of organism isolated from the specimen depends upon the type of hospital, local dominant flora and patient characteristics (gestational age, birth weight, postnatal age, associated conditions.[16]

The coexistence of osteomyelitis and septic arthritis in the newborn population is higher than in pediatric age group. This can be explained by the particular characteristics of the osteal blood supply of a neonate, in which contact between the metaphyseal and epiphyseal arteries promotes the rapid spread of infection and offers a channel for infection into the joint.[1] In our study, 26% of the infants had osteomyelitis or septic arthritis. 76% of the participants in our study acquired satisfactory outcomes. This is comparable to research by Akash et al. (73.3%) and Li et al. (72.9%).[2,7] Patients reported satisfactory results, according to Rudro et al. (43.6%) and Devi et al. (50%) reports.[1,15] Lee et al. reported poor outcome in 48% patients. However mean time to presentation from the onset of symptoms in this study was 4.32 days only. It is widely known that the location, age, length of the treatment, and the causative organism influence the course of septic arthritis in children.[8] Time of onset and delay in presentation and intervention is independent predictor of outcome in neonatal septic arthritis.[1]

Neonatal hip septic arthritis presentation is very non-specific, and is associated with poor prognosis, especially if the detection and intervention is delayed.[17] In our study those neonate who presented with hip joint involvement had unfavourable outcome then other joint involvement.

According to Usa et al.'s study, there is a clear correlation between poor outcomes and many joints involved, preintervention duration of less than seven days, culture-positive joint aspirate, and restricted range of joint movements at discharge.[1] As our sample was only 30,so predictor can't be measured. Thus, an early diagnosis and prompt referral, aspirate the joint fluid in all cases is the cornerstone for optimum results.

CONCLUSIONS

Most neonates with septic arthritis have a monoarticular involvement. The knee is the most often affected joint. Gram--negative bacteria, particularly Klebsiella, continue to dominate the bacteriological profile. We should focus on early referral to tertiary care facilities, rapid surgical intervention, and steps that assure optimal joint mobility at the time of discharge in addition to commencing intravenous antibiotics.

Limitation:

The follow-up observations were three times within 12 months age of the neonates. The sample size was small. Cases were refereed to other centers for orthopaedics consultation, that's why joint fluid study for all the affected joints are missing. Due to the severity of illnesses transportation was difficult and cost considerations during follow-up. Magnetic resonance imaging (MRI) or ultrasound (USG) could not be done in all patient.

Author Contributions:

Concept –RMC, KPD ; Design –RMC, KPD; Supervision- AM; Data Collection and/ or Processing- SA; Analysis and/ or Interpretation- RMC; Literature Search – RMC, KPD; Writing Manuscript- RMC, KPD; Critical Review- RMC, KPD, AM, SCM.

Conflict of Interest:

The authors have no conflict of interest to declare.

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Original Article

Pattern of Eosinophil and Neutrophil/ Lymphocyte Ratio in Different Stages of Chronic Kidney Disease

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Abstract

Various expensive interventions are used to diagnosis the different stages of chronic kidney disease (CKD). This research is aimed to determine the pattern of eosinophil and neutrophil/ lymphocyte ratio in different stages of CKD. Data was collected in January 2023 from the patients of CKD and from their files of reports. Permission was taken from the patients. The individuals were from civil hospital Karachi and they were the patients of CKD. Sample size was 200. There was irregular pattern of eosinophil and neutrophil/lymphocyte ratio in different stages of CKD. The eosinophil count and neutrophil/ lymphocyte ratio were higher in higher stages of CKD. The eosinophil and neutrophil/ lymphocyte ratio were higher in higher stages of CKD and the pattern was irregular. Study finds that eosinophil and neutrophil/ lymphocyte ratio were higher and the pattern was irregular in higher stages of CKD.

Keywords: *Chronic kidney disease, eosinophil, lymphocyte, neutrophil.*

INTRODUCTION

It has many types including type 1 and two, neonatal, gestational and maturity onset diabetes of young.[1] Diabetes is major risk factor of chronic kidney disease.[2] Neutrophil/ lymphocyte (N/L) ratio were high in CKD stage 4 and can be used as predictor in clinical practice.[3] Another research stated that N/L ratio was increased in CKD patients with ESRD in stage 4.[4] A source supported that eosinophils were high in CKD patients with granulocytes and there were low lymphocytes.[5] Another study conducted by Tariq, A et al showed that eosinophils were predictor of end -stage renal disease (ESRD) and progression of CKD.[6] Eosinophil count and neutrophil/ lymphocyte ration is used as marker for determine the severity of chronic kidney disease.[3- 7]

This research is implicated to determine the neutrophil/ lymphocyte ratio and eosinophil count pattern in different stages of CKD so hat early diagnosis can be possible without higher, expensive interventions

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MATERIAL AND METHODS

This cross-sectional study was conducted in the patients of CKD of civil hospital Karachi. The sample size was 200 and the information was taken from the patients and from their reports. Their participation was voluntarily. Inclusion criteria include diabetic patients with CKD. Exclusion criteria include non-diabetic patient with or without CKD. Descriptive percentages were calculated for variable. Chi square test was done.

RESULTS

Table I shows the stages of CKD, 12.2% respondents were having stage one, 5.6% were having stage 2, 34.2% were having stage 3, 24.5% were having stage 4 and 23.5% were having stage 5.

Table- I: Percentages of respondents in different stages of CKD

GFR staging	Percentages
Stage 1	12.2%
Stage 2	5.6%
Stage 3	34.2%
Stage 4	24.5%
Stage 5	23.5%

Table II contains the eosinophil count, 43.4% were having high eosinophil count, 6.1% were having low eosinophil count and 50.5% were having normal eosinophil count.

Table- II: Distribution of respondents having various eosinophil count

Eosinophil count	Percentages
High	43.4%
Low	6.1%
Normal	50.5%

Table III states that Neutrophil/lymphocytes ratio 25.5% were having high Neutrophil/ lymphocytes ratio, 2.6% were having Neutrophil/lymphocytes ratio and 71.9% were having normal Neutrophil/lymphocytes ratio.

Table- III: Distribution of respondents having different neutrophil/lymphocyte ratio

Neutrophil/lymphocytes ratio	Percentages
High	25.5%
Low	2.6%
Normal	71.9%

Table IV presents the eosinophil count pattern varies with different stages of CKD. There is no specific pattern and eosinophil count varies irregularly. Eosinophil count decreases from stage 1 to 2 and peaks at stage 3, then decreases from stage 3 to stage 4 and then again increases from stage 4 to stage 5. Eosinophil count peaks at stage 3 and is lowest at stage 2. Eosinophilia was observed in higher stages of CKD.

Table-: IV Relation of eosinophil and neutrophil/lymphocyte ratio at different stages

CKD staging	95% confidence interval of eosinophil count	P-value	95% confidence interval of neutrophil/ lymphocytes ratio	P-value
Stage 1	2.1-3.6	<0.001	1.5-3.2	
Stage 2	1.4-3.9		1.2-4.0	
Stage 3	6.1-8.4		2.1-2.8	0.027
Stage 4	3.7-5.6		2.4-3.1	
Stage 5	4.9-6.2		2.3-2.8	

Table V shows the neutrophil/ lymphocytes ratio pattern varies with different stages of CKD. There is no specific pattern and neutrophil/ lymphocytes ratio varies irregularly. At first it increases in stage one, then decreases at stage 2 and increases from stage 2 to stage 4 and then decreases in stage 5 (P=0.027). Stage 4 is associated with peak neutrophil/ lymphocytes ratio (<0.001) and stage 2 is associated with lowest neutrophil/ lymphocytes ratio (<0.001). Neutrophil/lymphocytes ratio was increased at higher CKD stages.

Table- V: Relation of different variable with eosinophil and neutrophil/lymphocyte ratio

Variable		Percentages	95% confidence interval of eosinophil count	P-value	95% confidence interval of neutrophil/ lymphocytes ratio	P-value
Gender	Male	33.7%	4.6-5.9	0.489	2.4-2.8	0.595
	Female	66.3%	4.8-6.8		2.1-2.8	
Age	<20	19.9%	4.3-8.6	0.007	1.9-3.0	0.340
	>50	32.7%	4.7-5.7		2.33-2.97	
	20-50	46.9%	4.6-5.8		2.31-2.90	
Do you smoke	yes	12.8%	3.2-5.6	0.01	1.8-2.8	0.395
	no	87.2%	5.05-6.2		2.4-2.8	
Do you have diabetes	yes	34.7%	4.7-5.8	0.602	2.30-2.89	0.712
	no	65.3%	4.8-6.3		2.34-2.87	
Do you have hypertension	yes	41.8%	5.5-6.3	0.01	2.3-2.8	0.278
	no	58.2%	4.2-5.9		2.2-2.8	

Table VI state the Regarding the age, >50 age group was associated with more severe CKD while <20 age group was associated with less severe CKD. Smoking was associated with less severe CKD while diabetes and hypertension were associated with severe CKD. Gender was not associated with CKD.

Table- VI: Relation of GFR with different variable

Variable		Percentages	95% confidence interval of GFR	P-value
Gender	Male	33.7%	4.8-6.8	0.489
	Female	66.3%	4.6-5.9	
Age	<20	19.9%	4.3-8.6	0.007
	>50	32.7%	4.7-5.7	
	20-50	46.9%	5.2-4.6	
Do you smoke	yes	12.8%	3.2-5.6	0.015
	no	87.2%	5.05-6.5	
Do you have diabetes	yes	34.7%	4.8-6.3	<0.001
	no	65.3%	4.7-5.8	
Do you have hypertension	yes	41.8%	5.5-6.3	0.001
	no	58.2%	4.2-5.9	

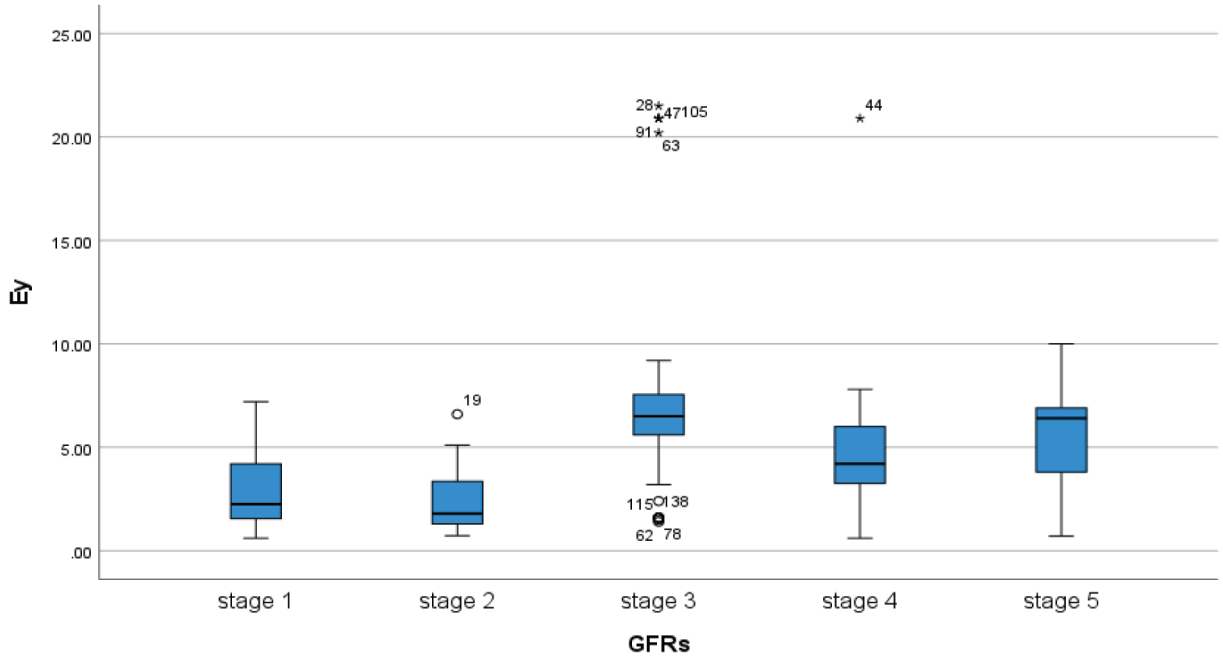

Figure- 1: *Eosinophil in different stages of CKD*

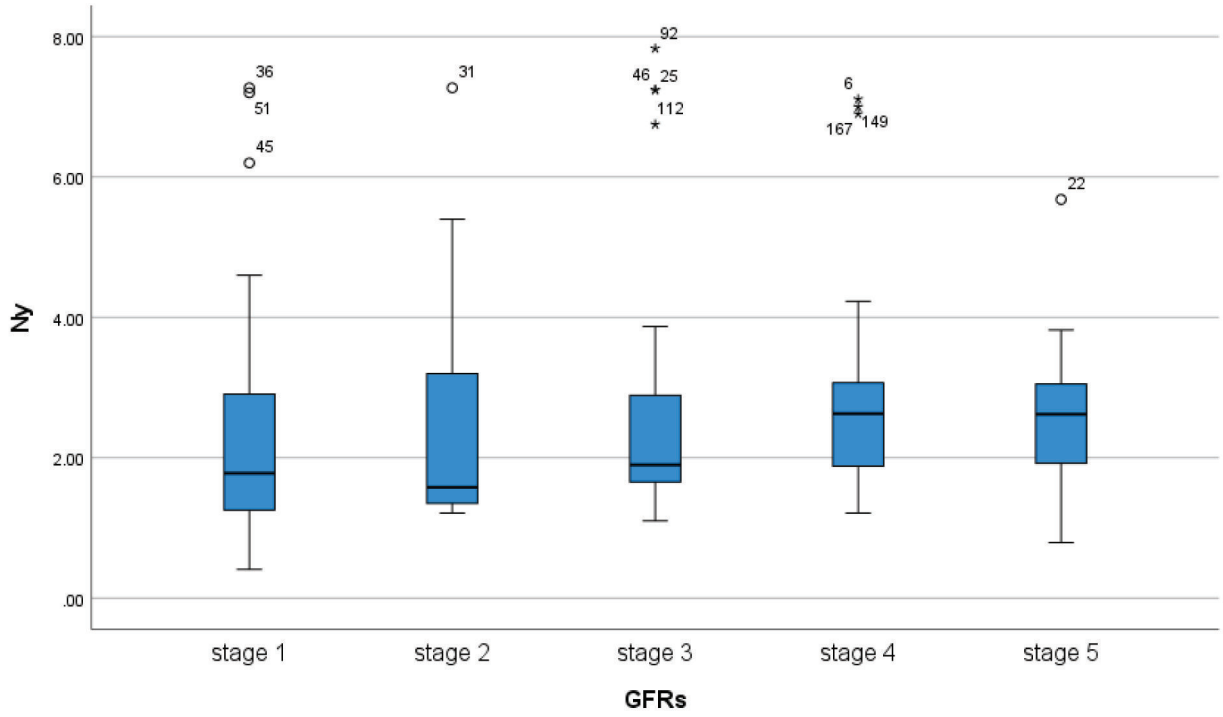

Figure- 2: *Neutrophil / eosinophil ratio in different stages of CKD*

Related to how different factors affect the eosinophil count in CKD patients, Age group 20-50 and >50 were associated with high eosinophil count in CKD patients while <20 age group was associated with low eosinophil count in CKD patients. Smoking was associated with low eosinophil count in CKD patients while hypertension was associated with high eosinophil count in CKD patients. Gender and diabetes was not associated with eosinophil count.

Related to neutrophil/lymphocytes ratio, gender, age, smoking, diabetes and hypertension were not significantly associated with neutrophil/ lymphocytes ratio in CKD patients.

DISCUSSION

A study conducted at southern Iraq by Khajehdehi P et al showed stages 1, 2, 3, 4, and 5 of CKD were found in 8.5%, 66.1%, 11.4%, 0.1%, and 0.1% of the participants, respectively[8]. A study conducted by Hill NR et al showed Stage 1,2,3,4,5 were 3.5%, 3.9%, 7.6%, 0.4% and 0.1% respectively.[9] A study conducted in Korea by Kim S et al showed prevalence of CKD according to stage were 2.0% stage 1, 6.7% stage 2, 4.8% stage 3, 0.2% stage 4, and 0.0% stage 5.[10] This research showed 12.2% respondents were having stage one, 5.6% were having stage 2, 34.2% were having stage 3, 24.5% were having stage 4 and 23.5% were having stage 5. This research percentages of CKD of different stages are greater than the Khajehdehi P et al, Hill NR et al and Korea by Kim S et al study. [8,9,10]

This study shows similar results with the study conducted by Tariq, A et al which showed that Eosinophilia was associated with ESRD and higher stages of CKD.[11] Another study conducted by Ishii R et al among cardiac patients showed the similar results regarding Eosinophilia being prevalent in higher stages of CKD.[12] Another study conducted by Rajiv Agarwal et al showed higher stages of CKD were associated with eosinophil.[13]

This research showed neutrophil/lymphocytes ratio was higher in higher CKD stages. This research shows similar findings to the following researches. Woziwodzka K et al who showed NLR was associated with higher stages of CKD patients.[14] A study conducted by Yoshitomi R et al in Japanese population showed NLR was greater in higher stages of CKD and predicts poor outcomes.[15] Solak Y et al [showed NLR showed a significant increase from stage 3 to stage 5.[16] Tonyali S et al showed NLR was positively correlated with CKD stage.[17] Altunoren O et al showed that NLR was greater in advances stages of CKD.[18] This

study contradicts with the study conducted by Yuan, Q et al who showed NLR was not associated with CKD progression.[19]

This study showed that severe CKD was associated with advanced age which is similar to the results of Nitta K et al [which showed ESRD was associated with older patients.[20] This research finding contradicts with the Ravani P et al research which showed that aging is associated with regression of CKD.[21] Wahsh HA showed that eGFR decreased with advancing age.[22]

This research shows that smoking was associated with less severe CKD which contradicts with the previous researches which are stated following. A research conducted by Yacoub R et al showed that smoking was associated with progression of CKD.[23] A study conducted by Xia J et al showed that smoking was independent factor related to progression of CKD.[24] Another study conducted by Yacoub R et al showed that smoking was associated with severe CKD and associated nephropathy.[23] A cohort study conducted among CKD Korean patients showed that smoking was associated with worsening of kidney function. Bundy JD et al [showed that smoking is associated with progression of CKD and mortality.[25] Jain G et al showed that smoking was associated with deterioration of kidney function but nicotine was less responsible for it.[26]

CONCLUSIONS

The eosinophil and neutrophil/ lymphocyte ratio were higher in higher stages of CKD and the pattern was irregular.

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Case Series

Thyroid Collision Tumour: Concurrence of Two Thyroid Malignancy

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Abstract

Thyroid carcinoma is the most common endocrine malignancy. However, it is exceptionally uncommon for two different thyroid malignancies to co-exist within the same thyroid gland. Herein, we present two cases where two different thyroid malignancies present as collision tumours of the thyroid gland. The hypothesis, epidemiology and management are discussed here. Case presentation: Case 1 was a 60 year-old woman presented with an accidental finding of left neck swelling. Ultrasound neck identified multinodular goitre with a suspicious left thyroid nodule. Ultrasound-guided fine-needle aspiration cytology of left thyroid nodule confirms papillary thyroid carcinoma. She underwent total thyroidectomy with central compartment neck dissection. However, histopathology reports a presence of medullary thyroid carcinoma and papillary microcarcinoma in the left thyroid gland. Case 2 was a 59 years old woman presented with left vocal cord paralysis with rapidly enlarging of a long-standing left thyroid swelling. Computed tomography identified bilateral thyroid swelling with left mass causing compression to the trachea and oesophagus. The patient subsequently underwent a total thyroidectomy bilateral central and left selective neck node dissections and left primary non-selective nerve re-innervation. A collision tumour with components of both Hurthle cell carcinoma and papillary microcarcinoma has been shown in histopathology. Collision tumours of the thyroid pose a diagnostic as well as a therapeutic challenge. They should be treated in a multidisciplinary team environment, and the care should be unique to the patient. The treatment should usually be directed by the most aggressive neoplasm.

Keywords: *Thyroid, collision tumours, hurthle cell, papillary microcarcinoma, medullary goitre*

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*For correcpondence

INTRODUCTION

Collision tumour is a term refers to when there is two or more tumour which are completely different in morphology and histology coexist in the same organ.In thyroid it is regarded as sporadic disease and has been documented through several literatures.[1] The commonest collision tumour in thyroid is the co-existent of medullary and papillary carcinoma but papillary thyroid carcinoma with Hurthle cell carcinoma is extremely rare[2]. We report two cases of collision tumor in thyroid and its associated management.[3, 4]

CASE PRESENTATION

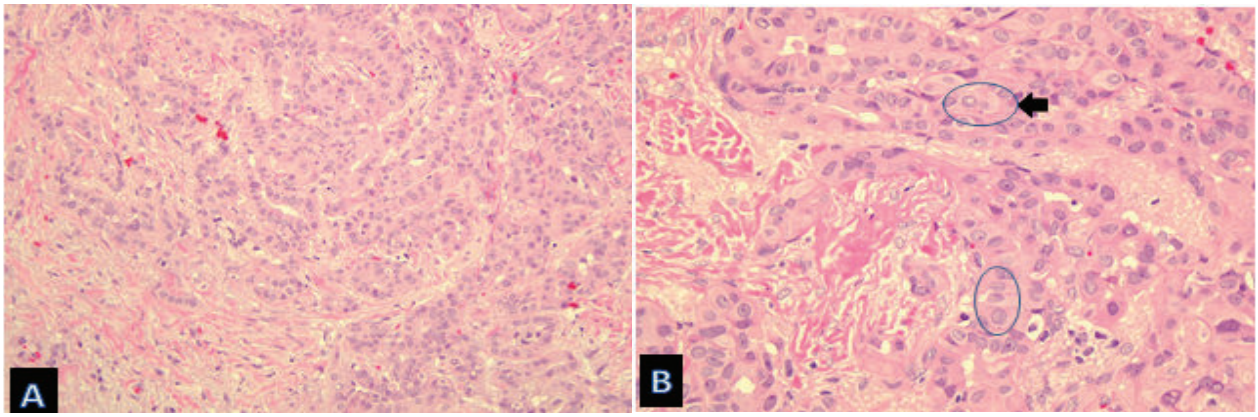
Case 1

A 60 year-old female presented with painless left neck swelling for one month. She denied any hyperthyroid or hypothyroid symptoms with no compressive symptoms. She had no previous history of irradiation to the neck and any known family history of thyroid cancer. On examination, she had left thyroid swelling measuring 1.5 cm with no palpable enlarged cervical lymph node.

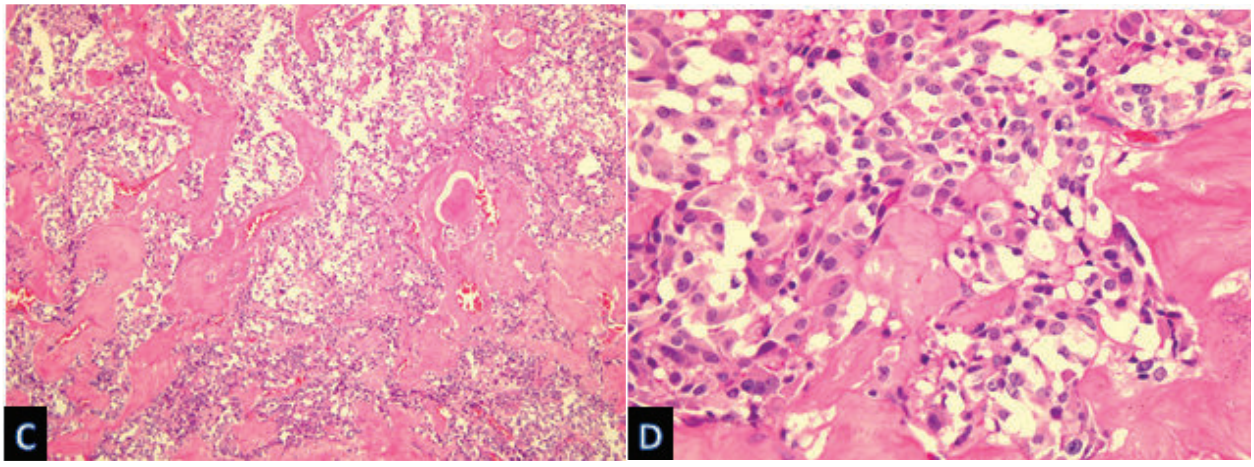
Thyroid function tests were normal. An ultrasound of the thyroid showed several multiple small nodules over both lobes with a suspicious nodule at inferior left lobe measuring 0.8 cm x1.2 cm. However, with no significant bilateral central and lateral cervical lymph nodes were detected. Ultrasound-guided fine-needle aspiration cytology yielded papillary thyroid carcinoma. She was counselled and agrees for total thyroidectomy with prophylactic central node dissection.

Histopathological examination of left thyroid lobe showed present of 3 nodules, largest 1.9 cm x 1.0cm x 1.0 cm at the center with cystic appearance filled with colloid and blood while the other two nodules measuring 0.7 cm x 0.5 cm x 0.3 cm and 0.6cm x 0.5 cm x 0.5 cm respectively at the opposite pole in the left lobe. Microscopic examination of the second nodule showed a neoplastic cell arranged in papillary structures with characteristic nuclei changes suggestive of papillary carcinoma. The third nodule revealed a tumor of neoplastic cells arranged in nests and trabeculae with amyloid suggestive medullary thyroid carcinoma. The cells were positive for calcitonin but not thyroglobulin. The central nodes had no metastasis and the right lobe is normal.

Her postoperative recovery was uneventful, with early postoperative serum calcitonin <2.00pg/ml (normal range 0.00-5.00 pg/ml), carcinoembryonic antigen 1.8 ng/ml (normal range <5.0 ng/ml), intact parathyroid hormone 3.1 pmol/L (normal range 1.1-7.3). She was later started on L-thyroxine. Her five year follow up show no recurrence.



Papillary Thyroid Carcinoma, Picture A*: The tumor composed of cluster of neoplastic cells arrange in papillary structure.* ***Picture B****: The cells have crowded oval nuclei exhibiting nuclear clearing, nuclear grooving and intranuclear pseudoinclusions. Circle with arrow show nuclear pseudo inclusion, circle show nuclear grooving*



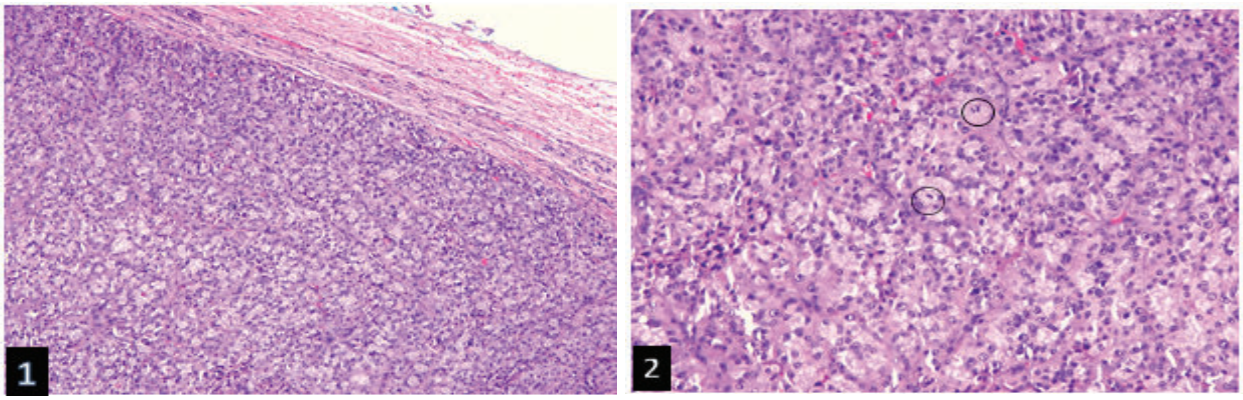
Medullary Thyroid Carcinoma, Picture C*: The tumour composed of neoplastic cells arranged in nests and trabeculae. The presence of amyloid material is confirmed by Congo red stain.* ***Picture D:*** *The cells have round to oval nuclei with conspicuous nucleoli and moderate amount eosinophilic cytoplasm.*

Case 2

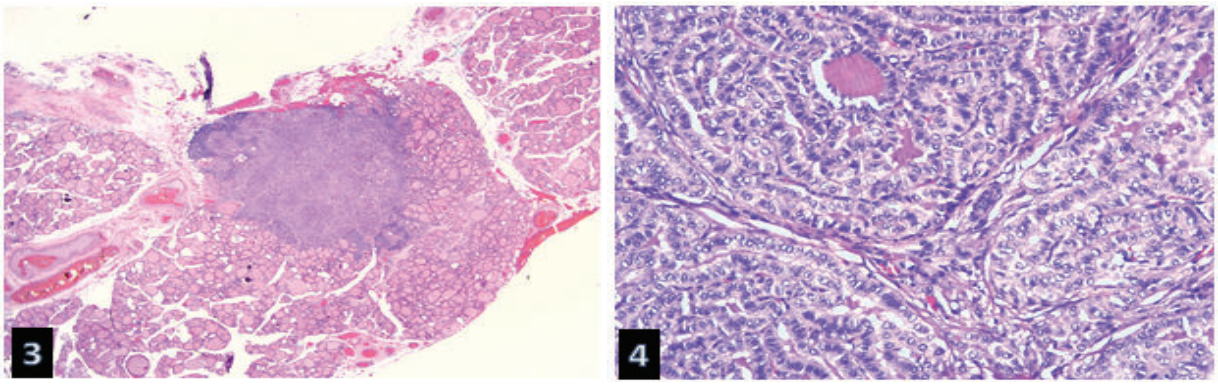
A 59-year-old lady was admitted to surgical ward for difficult in swallowing water and hoarseness of voice for the last one week. She had thyroid swelling for one year but since the last three months the swelling rapidly increase in size. She denied any hormonal thyroid symptoms, no previous irradiation to the neck and no family history of thyroid cancer. On examination, she had firm, 10 cm left thyroid nodule with palpable ipsilateral left cervical lymph nodes. A flexible laryngoscope revealed left vocal cord palsy. A CT scan of the neck and thorax showed the suspicious large had extended to the superior mediastinum with a compressed trachea and esophagus. The left internal jugular vein had thrombus within. There is no distant metastatic were observed. Fine-needle aspiration the nodule yielded Hurthle cell neoplasm. She underwent total thyroidectomy with bilateral central and left lateral lymph node dissection and left primary non-selective recurrent laryngeal nerve repair.

Histopathological examination of the left thyroidectomy specimen shows a capsulated tumour measuring 1.12 cm x 5.5 cm x 8.2 cm with invasion into perithyroidal tissue. The tumour composed of malignant cells infiltration arranged in solid sheets, pseudopapillary, microfollicular and trabecular growth pattern with vascular invasion. The cells are pleomorphic, exhibiting enlarged, vesicular nuclei with prominent nucleoli and voluminous eosinophilic granular cytoplasm: hence suggestive of Hurthle cell carcinoma. The right lobe had a whitish lesion over lower pole measuring 0.3 cm x 0.3 cm x 0.3 cm. It composed of follicles of varying sizes and papillary structures lined by neoplastic follicular cells exhibiting nuclear crowding, nuclear clearing with nuclear grooving and pseudo inclusion suggestive of papillary thyroid carcinoma. The central cervical group specimen had five lymph nodes and one of the lymph node had metastatic cells of papillary thyroid carcinoma.

The postoperative period of hospital stay was uneventful. The patient was subsequently referred for radio-iodine therapy.



Left thyroid gland *Figure 1: Malignant cells in left thyroid lobe arranged in solid sheets surrounded by fibrous capsule. Figure 2; The malignant cells are pleomorphic, exhibiting enlarged, vesicular nuclei with prominent nucleoli and voluminous eosinophilic granular cytoplasm. Mitotic figures in circle.*



Right thyroid gland *Figure 3: The tumour composed of follicles with varying sizes and papillary structures with fibrovascular core measuring 3mm in greatest dimension. Figure 4: Neoplastic follicular cells arranged in papillary structure with fibrovascular core exhibiting nuclear crowding, nuclear clearing with nuclear grooving and pseudoinclusion.*

DISCUSSION

Thyroid cancer ranked ninth for overall cancer incidence accounting for 567000 cases worldwide. It is frequently seen in women rather than men, with an incidence rate of 10.2 per 10000. Papillary thyroid cancer is the commonest thyroid cancer account for more than 90% of cases while[5] anaplastic carcinoma accounts for 2% of thyroid malignancy.[6,7] Even though differentiated thyroid cancers are the most common form of thyroid cancer, it is rare for them to have more than a single of malignancy existed concurrently within the same thyroid gland. The existence of two collision tumor in this case series has caught us by surprise in the cases of medullary thyroid carcinoma (MTC)-papillary thyroid microcarcinoma (PTMC) or Hurtle cell carcinoma (HCC)-papillary thyroid microcarcinoma (PTMC).

The criterial for collision tumor were first establish by Bilroth in 1879 and later redefined by Warren and Gates.[8] The criteria are: (a) each tumor provides a clear picture of malignancy, (b) each tumor must be distinct, and (c) it is necessary to eliminate the possibility that one of the tumors is a metastatic lesion from another. A combination of such malignancy occurring in the same gland or organ can be collision tumors, mixed tumors, or composite tumors.

A collision tumour is a coexistent independent tumour that is histologically distinct within the same organ or adjacent organ.[1] Collision tumours are different from mixed tumours, where there is a similar cell of origin for both tumours and distinguish from composite tumours, which contain two discrete cell populations.[10] However, the term had being used interchangeable in the literature. Collision tumours can happen in various organs such as the ovaries, lung, skin, colon, kidney, and stomach but are extremely rare in the thyroid.[11]

The occurrence of collision tumours is not well understood, but several hypotheses have been suggested, especially in explaining the coexistence of medullary carcinoma and papillary carcinoma. The first theory called random collision effect concerning the two primary tumours arose in continuity by accidental meeting with a random interaction related to an ambient alteration due to the same carcinogenic stimuli.[1] In this theory, changes in the thyroid gland for example due to radiation to the neck lead to changes in the environment in the thyroid gland that promote any primary tumor to growth independently. The second, hostage theory or neoplastic coercion is that the first tumour's growth alters the microenvironment, resulting in the second adjacent tumour development after the entrapment of normal follicular cells within the first tumor. However, this latter theory does not account for the rarity of collision tumours. The third theory called the stem cell theory when indicated a similar stem cell of origin for the two primary tumours.[12] It happen when the stem cell had the ability to transform into two different type of tumor in the same organ.[12]

Collision tumour of the thyroid gland shows female predominance with a mean age of incident 53.4 years.[2] This finding is similar to the general population of patient being diagnosed with single thyroid malignancy. An anterior cervical mass in 90% of cases is the initial presentation of the tumours. The majority of the patient has metastasis during presentation toward cervical node and often a mixed combination of the two collision tumour in about 36% of cases. It found that fine-needle aspiration cytology rarely detects collision tumour presence and the majority of the cases diagnosed from the histological finding after thyroidectomy. The most common collision tumour is medullary and papillary carcinoma and its accounts for 60% of cases, followed by papillary and squamous cell carcinoma.[2] There is only two other reported case of Hurthle cell carcinoma – papillary carcinoma combination reported in the literature, with this cases adding to the number of cases reported.[3,4] A population-based study has placed the incidence of differentiated thyroid cancer, and medullary cancer coexisted in 12.3% of all medullary cancers with incidence is increasing.[13] Patients with coexisting medullary thyroid carcinoma and differentiated thyroid carcinoma have also been shown to be diagnosed sooner in tumor growth than patients with medullary thyroid carcinoma alone, leading to a better prognosis. They also conclude that medullary thyroid carcinoma tend to impact patient survival more than differentiated thyroid cancer.[13]

The management for collision tumour of the thyroid possesses a unique challenge due to the presence of the dual pathology in the tumour and rarity of the disease. The treatment must involve a multidisciplinary approach, and patient-specific with the most aggressive tumour should be the target treatment. Most collision tumours received multimodal treatment in the literature, involving surgery and adjuvant treatment. In about 27% of the collision tumour, will receive total thyroidectomy, neck dissection and radioactive iodine.[2] The survival data for a patient with collision tumour is very limited owing to the rarity of the

disease; however, Ryan N et al. suggested that the metastatic and survival rates are consistent with matched single thyroid pathology.[2]

CONCLUSIONS

Collision tumor of thyroid is a rare condition with scarce literature case series reported. It poses management challenges for these tumors. As no clear hypothesis is able to describe clearly the pathogenesis of this tumor, a combination of several hypotheses must be embraced for better understanding. The best management of this tumor should be based on the multidisciplinary team decision with the most aggressive tumor should be the target treatment.

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Case Report

Maternal Atrioventricular Nodal Re-entrant Tachycardia During Pregnancy: Case Report

*Podder P[1], Saha J[2]

Abstract

During pregnancy, the physiological changes predispose a woman for the development of new-onset or recurrent arrhythmia. Atrioventricular nodal re-entrant tachycardia (AVNRT) is a common supraventricular tachycardia (SVT) in reproductive age of woman. Although often it is benign in nature but concerning. Electrical cardioversion is safe during pregnancy. Electrophysiological study (EPS) and Radiofrequency ablation (RFA) can be performed in selected patients. Hereby, we report a case of a woman in her second trimester of pregnancy presented with diagnosed case of AVNRT. She was monitored by multidisciplinary team. Electrocardiogram (ECG) and Echocardiogram reveals normal changes. EPS and RFA was done one year back at the time of diagnosis. At her 37 weeks of pregnancy, she developed scar tenderness. Emergency caesarean section was done in presence of cardiologist. Maternal and perinatal outcome were good.

Keywords: *Atrioventricular nodal re-entrant tachycardia, cardio-obstetrics, catheter ablation, radio frequency ablation, supraventricular tachycardia.*

INTRODUCTION

Atrioventricular Nodal Re-entrant Tachycardia (AVNRT) is a prevalent form of supraventricular tachycardia (SVT) that is frequently observed in women of childbearing age. This SVT is characterized by a regular and rapid heart rhythm and happens due to the creation of a reentry circuit specifically within the atrioventricular (AV) node and the adjacent atrial tissue. AVNRT is sorted as a paroxysmal SVT (PSVT) because of its sudden onset and termination. It is more common in women compared with men.[1-3] AVNRT can present at any age, but as with AV reentrant tachycardia (AVRT) that involves an accessory pathway, it is more likely to begin in young adults.[1] There are no reliable data on the incidence in pregnant women. In the general population, the occurrence rate is 35 cases per 100,000 person-years.[2] Over half of these patients are asymptomatic.

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*For correspondence

The main mechanism for the development of SVT is via reentry (atrioventricular nodal reentrant tachycardia in 60 % of cases and atrioventricular reentrant tachycardia in 30 % cases. [3] The QRS complex in AVNRT is usually narrow (≤120 milliseconds), reflecting normal ventricular activation through the His-Purkinje system. However, during SVT, tachycardia with a broadened QRS complex can occur due to rate-related abnormal conduction or the presence of an underlying bundle branch block.

Nonetheless, during supraventricular tachycardia (SVT), irregular conduction related to heart rate or an existing bundle branch block can lead to a tachycardia characterized by a wider QRS complex. Incidents of SVT become more common, especially in the third trimester of pregnancy. This increased prevalence may be attributed to several proposed mechanisms, including the hyperdynamic circulation, hormonal changes, elevated levels of catecholamines in the bloodstream, heightened sensitivity of adrenergic receptors, and the expansion of the maternal effective circulating volume, which in turn stretches the atria.[4,5] Potential risk factor in pregnancy is underlying congenital or structural heart disease.[6] In most cases, there is no history of heart disease.

For hemodynamically stable patients, initial treatments such as sinus carotid massage or Valsalva maneuvers are attempted, followed by the administration of appropriate medications. If these measures prove ineffective, or if there is hemodynamic instability, more aggressive interventions, including electrical cardioversion or invasive procedures like radiofrequency ablation, may be necessary. However, it's essential to consider that these interventions carry potential risks for both the mother and the developing fetus. It's worth noting that there is a lack of comprehensive large-scale studies or randomized control trials that assess the safety of electrical cardioversion in pregnancy or identify optimal agents for achieving successful cardioversion in this specific population.[7]

We present a diagnosed case of maternal AVNRT with hemodynamically stable with good maternal and fetal outcome.

CASE REPORT

A 27 year-old female G3P2 came at 1st time at her 26 weeks of pregnancy for antenatal checkup. She was a diagnosed case of AVNRT. At2022, She complaints of sudden onset palpitations, uneasiness and dull aching chest pain. She had no prior medical history of significant illnesses, particularly any conditions related to the heart or lungs. She was admitted, and the clinical examination showed a pulse rate of 220/min with electrocardiogram (ECG) showing presence of Atrioventricular nodal re-entrant tachycardia (AVNRT) but with hemodynamic stability (BP-140/70 mm of Hg). Anemia and hyperthyroidism were excluded. Cardiology consultation was taken. ECG and Echocardiogram was done and AVNRT was diagnosed. Then decision of EPS and radiofrequency ablation was taken and done successfully. Patient was reverted to normal sinus rhythm with the help of IV adeno-sine at that time.

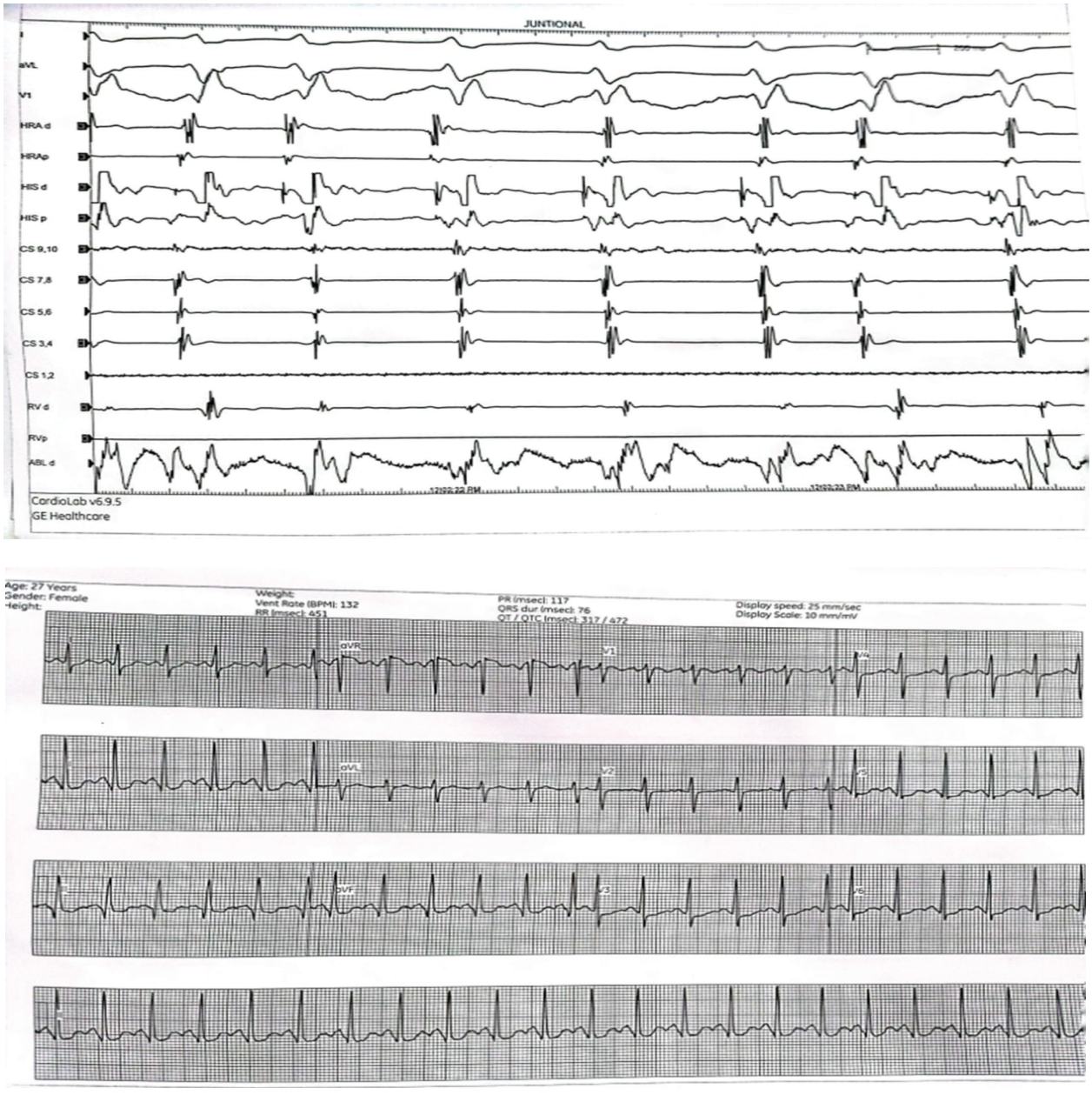

Figure 1: ECG showing AV nodal reentant tachycardia

She was in regular antenatal checkup. Cardiology consultation was taken also regularly and suggested to take propanolol daily for 12 hourly. She has history of previous two caesarean section. At her 37 weeks of pregnancy she developed tenderness over the scar. Pulse was 115 b/min and Blood pressure was 120/70 mm of Hg. Emergency Caesarean section was performed. Cardiology consultation was taken and ECG was done.

On follow-up, patient and neonate, both were stable. No repeat episodes of SVT observed and discharged on her third postoperative day with advice.

DISCUSSION

The mechanism of increased arrhythmia increases burden during pregnancy is unclear, but it is likely because of a combination of hemodynamic, hormonal and autonomic changes. Increases in effective circulating blood volume of 30% to 50% are seen beginning at 8 weeks of gestation and peaking at 34 weeks.[8]

The occurrence of SVT during pregnancy poses risks to both the mother and the developing fetus. Exacerbate symptoms of SVT are shortness of breath, palpitations, dizziness and presyncope. Clinical assessment of vital signs and 12-lead ECG investigation are mandatory or an accurate diagnosis of arrhythmia.[7,9] Echocardiography is indicated to exclude structural and functional heart diseases. Co-existence of organic heart diseases is an important risk factor for arrhythmias during pregnancy.

Early consultation with a cardiologist is advisable for the diagnosis of SVT and the identification of any potentially life-threatening underlying causes. Close collaboration between the cardiologist and the obstetrician is important throughout the pregnancy as well as puerperium to develop care strategies for potential recurrences of SVT.[3,7,9] In our case along with cardiologist we continue the treatment.

Effectively managing SVT during pregnancy presents a complex clinical dilemma. The decision-making process should carefully weigh both maternal and fetal factors. Continuous monitoring of both the mother and the fetus should be maintained throughout acute treatment.

As per the 2019 guidelines for the management of individuals with supraventricular tachycardia, vagal maneuvers are recommended as the initial treatment approach for AVNRT in pregnant individuals. It is also recommended to avoid the use of antiarrhythmic drugs in pregnant women with mild symptoms or rare and short episodes of arrhythmia.[9] If symptoms are present and the arrhythmia is not tolerated by the woman, and if periodic disturbances in uteroplacental flow are present, treatment with a cardioselective beta-blocker should be considered, preferably after the first trimester of pregnancy. If there is no improvement after the treatment, the substrate of the arrhythmia may be ablated, preferably after the end of pregnancy. [9,10]

Catheter ablation should be considered for arrhythmias detected prior to pregnancy to avoid arrhythmia exacerbation and the need for medical suppression during pregnancy.[11] Catheter ablation can be considered during pregnancy in cases of arrhythmia refractory to medical therapy, but this should only be considered in a center with experienced operators, and with strong consideration of a non-fluoroscopic approach, if available.[12-14]

CONCLUSIONS

Multidisciplinary approach, regular follow-up, prompt and correct diagnosis, proper use of physiological treatment and appropriate drugs can save the pregnant mothers from atrioventricular nodal re-entrant tachycardia and as well as the life of fetus.

CONFLICT OF INTEREST STATEMENT

All authors confirm that they have no conflicts of interest to disclose. Patient has given consent for publishing photograph, clinical history and management of the same and was assured that anonymity will be preserved.

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Obituary news January 2023

BMA would like to express deep condolence on deaths of the following notable physicians in recent past:

Sl. No.	Name	Date of Death
1	Dr. Sharmin Sultana	09/09/2022
2	Dr. Chemon Ara Begum	18/09/2022
3	Dr. Samina Rahman	27/09/2022
4	Dr. Monjur Morshed	03/10/2022
5	Dr. Asad Shikder	16/10/2022
6	Professor Dr. Quazi Hamid Asgar	27/10/2022
7	Dr. Mujahidul Islam Abir	27/10/2022
8	Dr. Sabiha Rahman Sathi	29/10/2022
9	Lt. Col. Dr. Shafiqul Hasan	31/10/2022
10	Professor Dr. Md. Abdul Hannan	31/10/2022
11	Dr. Sawkat Ali Laskar	03/11/2022
12	Professor Dr. A S M Fazlul Karim	03/11/2022
13	Dr. Rabiul Islam	13/11/2022
14	Dr. Abdul Kader Jasim	17/11/2022
15	Dr. Karim Rezwan Hasan	18/11/2022
16	Dr. Mahbub Alam	23/11/2022
17	Professor Dr. Hasan Md. Abdur Rauf	24/11/2022
18	Dr. Saiful Islam Kanchan	27/11/2022
19	Dr. Akter Hosen	08/12/2022
20	Dr. Yousuf Ahmed Rubel	09/12/2022
21	Dr. Tofazzal Hosen	19/12/2022

May Allah bless the departed souls.
Our heartiest commiseration to the deceased's family, our prayers are with them during this difficult moment of their life.

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